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VOLUME 85 NUMBER 5

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

November, 1949

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

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BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

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This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

EVIDENCE FOR THE PRESENCE OF RIBONUCLEIC ACID IN THE CYTOPLASMIC BODIES THAT APPEAR IN THE HEPATIC AND ADRENAL EPITHELIAL CELLS OF MAN IN ACUTE INFECTION

ARNOLD R. RICH AND MORGAN BERTHRONG

From the Department of Pathology, The Johns Hopkins University Medical School

Received for publication August 11, 1949

Not infrequently there appear in the epithelial cells of the liver and adrenal cortex of man strongly basophilic cytoplasmic bodies of undetermined nature and significance. They vary in size and shape, some having a globular form, some appearing as straight or curved rods, and some having an irregular contour; their size ranges from less than a micron to several micra in diameter or length. They may be present sparsely or in profusion, and may be scattered indiscriminately in the cytoplasm or arranged in rows or palisades that lie along the margins of the cells.

It is strange that these cytoplasmic bodies in the human liver have been so generally overlooked and neglected, considering the frequency of their occurrence in pathological material, and their often striking appearance in the routine hematoxylin-eosin stain of tissue fixed in the ordinary fixatives. They receive no mention at all in treatises, text-books or systems dealing with the pathology of the liver or adrenals. For some years we have been impressed by their relation to severe infection, and that impression was confirmed by a study carried out in this laboratory several years ago by Santee (2), who found that in a series of unselected cases in which the liver and adrenals were examined histologically, the presence of the cytoplasmic bodies was associated with acute infection in the great majority of cases. While we certainly do not intend to imply that infection is the sole condition under which these bodies occur, it seems nevertheless clear that some factor associated with infection provides a particularly favorable stimulus or condition for their appearance.

In the course of a study of the relation of certain cellular constituents to the functional and reproductive potentialities of the liver cells (1), it became desirable to examine more closely the nature of the cyto-

plasmic bodies that form the subject of the present paper. The purpose of this report is to present evidence for the presence of ribonucleic acid in these bodies.

The possibility that the bodies in question might contain ribonucleic acid was suggested in the first place by the fact that they are markedly basophilic and, as is now recognized, cytoplasmic basophilia is often associated with the presence of ribonucleic acid. In the second place, these bodies bear some resemblance to the basophilic, so-called "protein storage" bodies of Berg (3), which are present normally in the liver cells of various animal species when the diet contains an adequate amount of protein, and several investigators have presented evidence for the presence of ribonucleic acid in the latter bodies in the hepatic cells of the normal rat (4, 5, 6, 15), an observation that we have confirmed. In a study of the nature of the basophilic bodies that become prominent in the human liver under pathological conditions, and of the factors responsible for their development, the present examination of their possible nucleic acid content was carried out.

As long ago as 1913, van Herwerden (7), in a pioneering study, demonstrated that certain basophilic cytoplasmic structures could be removed from animal cells by immersing the sections in a solution of "nuclease" prepared from spleen, and offered the observation as evidence for the presence of nucleic acid in those structures. In 1920, Walter Jones (8) prepared from pancreas a heat-stable enzyme that depolymerized ribonucleic acid, but not desoxyribonucleic acid; and in 1938 Dubos and Thompson (11) obtained from pancreas and other tissues a heat-stable enzyme, doubtless identical with that of Jones, which depolymerized ribonucleic acid and did not affect any other substrate, including desoxyribonucleic acid, against which it was tested. The latter investigators termed the enzyme "ribonuclease." Various observations had indicated a relationship between cytoplasmic basophilia and the presence of ribonucleic acid, and Brachet (9), in 1940, found that the enzyme prepared by the method of Dubos and Thompson removed cytoplasmic basophilia from various cells, whereas the nuclear basophilia, associated with the presence of desoxyribonucleic acid, remained unaffected. This observation has been confirmed by subsequent investigators in studies of various types of cells. We therefore sought to determine whether the bodies under present

consideration are susceptible to the action of ribonuclease, as a test of their possible ribonucleic acid content.

We have found, as might be expected, that the enzyme when insufficiently purified is unsuitable for the specific histochemical demonstration of ribonucleic acid, apparently because of contamination with other enzymes which affect cellular constituents other than ribonucleic acid. In the experiments reported here, therefore, crystalline ribonuclease (Worthington Biochemical Laboratory, Freehold, N. J.) was used throughout. While crystallization is, of course, no guaranty of chemical purity, this crystalline product is demonstrably free from sources of error that become obvious when a less purified preparation of the enzyme is used.

The procedure adopted was as follows: Material from autopsies on cases of severe infection, in which the liver and adrenals exhibited well-preserved basophilic cytoplasmic bodies in the routine hematoxylin-eosin sections was used for study. The tissues had been fixed in Helly's fluid and embedded in paraffin. Freshly cut sections were deparaffinized in the usual manner, the precipitate of mercury salt from the fixative was removed with iodine followed by sodium thiosulfate, and the sections were thoroughly washed in tap water followed by distilled water. Slides having a large well for the test fluids were prepared by affixing two parallel strips of glass, cut from a microscopic slide, to the surface of another slide, with balsam or Duco Household Cement, or by fusion in a glass-blower's flame. The glass strips were placed three or four centimeters apart, leaving a space between them adequate to accommodate the length of the section of tissue. To facilitate handling, the under surface of each slide so prepared was cemented to a second slide, one end of which projected about two centimeters beyond the corresponding end of the upper slide.

A solution of crystalline ribonuclease in veronal acetate buffer was made up freshly immediately before each experiment, in the proportion of 1 mg. of the enzyme to 1 cc of buffer. The buffer was prepared by adding 51 parts of M/10 veronal to 49 parts of M/10 acetic acid. The pH of the buffer, as determined with a Beckman glass electrode pH meter, varied between 6.75 and 6.79.

In the case of each section to be subjected to the enzyme, the ribonuclease solution was placed in the well of a prepared slide, and

the slide bearing the deparaffinized, washed section was inverted and placed upon the glass strips that formed the ends of the well. All air bubbles were removed, and more enzyme solution was added with a capillary pipette until the entire well was filled and the tissue section completely bathed in the solution. In each case a control was prepared by exposing a tissue section from the same block to the veronal buffer alone in the same manner. The test and control preparations were placed in moist chambers in an incubator at 37° for two to five hours, after which the slides containing the tissue sections were washed thoroughly in running tap water, then washed for 5 minutes in each of three changes of distilled water and, finally, were stained with Giemsa, by which the cytoplasmic bodies in question, in their unaltered state, are stained a deep purple.

It was found that whereas the basophilic bodies were unaffected by contact with the buffer alone, they were no longer stainable or visible in the cells that were exposed to the solution of ribonuclease, indicating that the enzyme had depolymerized a ribonucleic acid component of the bodies, to which their basophilia is due. The desoxyribonucleic acid of the nuclear chromatin was unaffected by the ribonuclease, and the nuclei of the cells exposed to the enzyme retained their property of becoming brilliantly stained with Giemsa.

The property of trivalent lanthanum ions to combine with nucleic acids, forming compounds of very low solubility, was applied by Caspersson (10), in 1936, to render cellular nucleic acids in tissue sections insoluble, so that they would remain "precipitated" *in situ* when the tissue section was exposed to the action of digestive enzymes. By this method he was able to study more accurately the localization of nuclear nucleic acids. Opie and Lavin (15) subsequently used this method to render cytoplasmic ribonucleic acid insusceptible to the action of ribonuclease. We therefore examined the effect of lanthanum upon the cytoplasmic bodies under present consideration. Sections of liver and adrenal, the cells of which contained these bodies, were deparaffinized and placed in a 0.2 M solution of lanthanum chloride ($LaCl_3 \cdot 7H_2O$) for three hours at room temperature, after which they were washed for 30 minutes in running tap water, followed by two changes of distilled water. Control sections, from the same blocks, were treated in the same manner with 0.6 M sodium chloride, instead

of lanthanum chloride. The sections were then exposed to ribonuclease in the manner described above. Giemsa stains showed that whereas the ribonuclease completely removed the basophilic bodies from the control sections, the bodies in the lanthanum-treated sections were definitely protected against the action of the enzyme, staining somewhat less intensely with Giemsa than control sections not exposed to the enzyme, but staining with clarity. This is additional evidence for the presence of ribonucleic acid in the cytoplasmic bodies under consideration.

Since it has been shown by Dubos and MacLeod (12) that bacteria can be robbed of their Gram-positivity by exposure to ribonuclease, indicating that ribonucleic acid is an important component of the bacterial substance responsible for Gram-positivity, sections of liver and adrenal containing the cytoplasmic bodies were stained with the Gram stain. The bodies were not Gram positive. This negative result is, of course, no valid evidence against the presence of ribonucleic acid in the bodies, for it is clear that the Gram-positivity of bacteria depends not solely upon the presence of ribonucleic acid, but also upon certain other factors that are not understood at present, among them a physical factor; for the mere physical disruption of Gram-positive bacteria suffices to render the residue Gram-negative in spite of its content of ribonucleic acid, recalling the loss of acid-fastness by tubercle bacilli that are mechanically traumatized (16).

DISCUSSION

The functional significance of the basophilic, cytoplasmic bodies that are at times so strikingly apparent in the epithelial cells of the liver and adrenal cortex of man, and which were the object of the present study, is not yet understood. We have not encountered them in the cells of other glandular organs, even in cases in which they were brilliant and abundant in the liver and adrenals.

It seems clear, from our observation of these bodies in pathological material over a period of years, that severe acute infections provide especially favorable conditions for their appearance. Indeed, the association is so close that when these bodies are prominent in the liver cells we have become accustomed to suspect a concomitant acute infection, and have learned that there is a high degree of probability

that that surmise will be correct, though infection is certainly not essential for their appearance, and they may be inconspicuous, faint or absent in cases of severe infection. In some of the latter instances their absence is probably the result of post-mortem changes that lead to the disappearance of the bodies. They appear to be highly susceptible to autolysis, and every degree of lysis, from loss of capacity to stain well to complete disappearance, can be seen in cases in which postmortem autolysis is in progress. Their fading and disappearance after the death of the cell is also obvious in freshly dead cells in foci of necrosis in livers in which they are abundantly present and brilliantly stained in the neighboring non-necrotic cells. Since, in routine autopsy material, the adrenal cortical cells commonly show more advanced autolytic changes than the hepatic cells, the bodies are, correspondingly, seen less often and may be less brilliantly stained in the adrenals than in the liver. Furthermore, it is of interest that the number and size of the bodies in the adrenal cortical cells bear an inverse relation to the amount of cortical lipoid. The bodies are ordinarily absent from cells that are heavily laden with lipoid, even when they are present abundantly and brilliantly in the adjacent lipoid-free cells of the same section.

The present studies provide evidence that ribonucleic acid is an important constituent of these cytoplasmic bodies. In the first place, the bodies are basophilic, and there is much accumulated evidence that cytoplasmic basophilia is commonly associated with the presence of ribonucleic acid; in the second place, ribonuclease, which depolymerizes ribonucleic acid, robs the bodies of their stainability by basic dyes; and in the third place, lanthanum salts precipitate ribonucleic acid, and treatment with lanthanum chloride protects these bodies against the action of ribonuclease.

Nothing can be stated with assurance at present regarding the functional significance of these bodies. While they bear some resemblance to the familiar "protein storage" bodies described by Berg (3) in the normal livers of lower animals, as was stated above, and although both types of bodies contain ribonucleic acid, it would be premature to conclude that they are identical chemically and functionally. In the consideration of this point it is necessary to recall that the "protein storage" bodies are, with regularity, prominent under normal conditions in the hepatic cells of the animal species in which they occur, if

the diet has been adequate in protein. The bodies with which we are concerned, on the contrary, are certainly not present, in the form described here, with the same regularity under normal conditions, if, indeed, they ever occur normally. We have examined with care sections of the liver and adrenals from twelve unselected cases in which death occurred, in otherwise essentially normal individuals, either suddenly or within several hours following the precipitating cause. In four of the cases death resulted from trauma (autopsy nos. 14822, 17724, 18153, 18933); in four, from rupture of a congenital aneurysm of a cerebral artery (autopsy nos. 15513, 17589, 18094, 19426); in two, from suicide (autopsy nos. 19988, 21692); in one, from anesthesia during an operation for myoma of the uterus (autopsy no. 18459); and in one case, a young man apparently in perfect health until he died suddenly, no cause of death was found at autopsy (autopsy no. 16312). While in several of these cases there were in the liver cells, small aggregates of cytoplasm which, when the cells were stained rather heavily with the basic dye, exhibited a very faint basophilia, the basophilia was scarcely, if at all, more marked than that of the remainder of the cytoplasm, and far from the striking basophilia of the bodies under consideration. In none of these cases were cytoplasmic bodies of the type under consideration detectable. If these bodies occur at all under normal conditions in man, they must be extremely sparse, inconspicuous and of irregular occurrence. Under certain pathological conditions, however, they appear in profusion and with striking prominence. It would appear, therefore, that if they do exist in the normal liver and adrenal of man, they must exist for the most part either as minute forms that are virtually invisible in ordinary preparations, and which become greatly enlarged, with a marked increase in ribonucleic acid content, under pathological conditions; or else they must exist in some precursor and different form, with different staining properties. In being absent, or infrequent and of very irregular occurrence under normal conditions, they differ from the "protein storage" bodies of the liver cells of lower animals.

Another difference resides in the fact that these bodies, when they are present in the liver, are commonly found, and often in profusion, in the cells of all zones of the adrenal cortex also; whereas the "protein storage" bodies in the livers of normal lower animals are not accompanied by the presence of similar bodies in the adrenal cortex.

In this connection, it is of particular interest that we have found

cytoplasmic bodies of precisely the same appearance, staining properties and susceptibility to ribonuclease, in the adrenal cortical cells in the absence of infection, in two cases of *reticular zone hyperplasia associated with the adrenogenital syndrome*. The basophilic bodies in these cases were confined to the hyperplastic reticular zone cells. In one case (Autopsy No. 16090), these cells had crowded out virtually all of the cells of the zona fasciculata and zona glomerulosa, and the patient died from cortical insufficiency. The bodies were abundant in the hyperplastic reticular zone cells. None were present in the liver. In the other case (Surg. Path. No. 49-838), the proliferation of the reticular zone cells had replaced the zona fasciculata, but had left the zona glomerulosa intact. The hyperplastic reticular zone cells contained many basophilic bodies, but none were present in the cells of the zona glomerulosa. The liver was not available for study in this case. The adrenal had been removed surgically in an attempt to ameliorate the condition of the patient.

While, therefore, it is possible that the "protein storage" bodies and those under present consideration may be functionally identical, it would seem proper, in view of the apparent differences, to study each independently until further information establishes their identity or non-identity. Even if they prove to be identical it is worth pointing out that, although the bodies found normally in the lower animals have been termed "protein storage" bodies because of the assumption that they represent simply a reserve, emergency store of body protein, and while their presence is demonstrably dependent upon the ingestion of protein, their actual functional significance is not really understood. In fact, there are good reasons for regarding it as more probable that they represent loci of enzyme activity or enzyme synthesis, rather than a mere reserve store of protein. A variety of enzymes have been found in Claude's (13) "large granule" cytoplasmic fraction of the rat's liver cells, and that fraction is also rich in ribose nucleoproteins. It is true that the large granule fraction, which should contain the "protein storage" bodies, also contains the mitochondria, and the precise localization of the enzymes in the different types of granules is not yet established. At all events, the fact that the so-called "protein storage" granules shrink and even disappear when protein is withheld from the diet is no proof that these granules are merely reserve stores of protein,

as has been assumed from this fact. The same effect of a protein-free diet could be expected if the granules represent loci of enzymes for, in the absence of dietary protein, the daily reparative synthesis of the protein components of enzymes would be expected to be impaired, with a corresponding progressive diminution in the size of any particulate enzyme aggregate. Indeed, Miller (14) has presented evidence that during a seven day fast the catalase, alkaline phosphatase, xanthine dehydrogenase and cathepsin activity of rat liver decrease together with the loss of liver protein, and that on a low protein diet there occurred a decrease in the one hepatic enzyme studied (arginase).

Do the basophilic bodies which become prominent in the human liver and adrenal under pathological conditions represent a degenerative phenomenon, or an expression of an increased activity of some normal function of the cells? We believe that there are reasons for regarding the latter as the more probable. When they become prominent during infection, to what particular disturbance caused by the infection is their appearance a response? It is obvious that the isolated effect of each of a variety of disturbances that infections bring into being can be studied in human pathological material in cases in which those individual disturbances were produced by causes other than infection, and studies of this nature in relation to the bodies under consideration are at present in progress in our laboratory, as is also an experimental investigation of other aspects of the chemical nature of the bodies, the factors responsible for their development, and their possible relation to recognized normal cytoplasmic constituents, such as mitochondria.

CONCLUSIONS

Under certain pathological conditions there appear in the cytoplasm of the epithelial cells of the human liver and adrenal cortex sharply outlined, strongly basophilic bodies, the existence of which has been generally overlooked. Severe acute infections appear to provide a particularly favorable stimulus for their development in both liver and adrenal.

These bodies become prominent, also, in the adrenal in association with hyperactivity of the cortical reticular zone accompanied by the adrenogenital syndrome.

The full range of conditions under which they occur, or increase in prominence, has not yet been defined.

Exposure to crystalline ribonuclease, an enzyme which depolymerizes ribonucleic acid, robs these bodies of their basophilia, and results in their disappearance in stained sections, indicating that they contain ribonucleic acid. This is further indicated by the fact that treatment with lanthanum chloride, which renders ribonucleic acid insoluble, protects the bodies against the effect of subsequent exposure to ribonuclease.

The nature of the residual chemical components of these bodies, their functional significance, and the precise conditions responsible for their appearance, are problems for further investigation.

We wish to acknowledge the helpful suggestions given us during the course of this work by Dr. Norman Weissman.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Miss Joan Wheaton)

All sections were stained with Giemsa; all photographs were taken at the same magnification.

FIG. 1. Liver from case of lobar pneumonia with abscess formation (Autopsy No. 21639). The section was treated with veronal buffer before staining. The cells contain many basophilic bodies.

FIG. 2. Section of liver from same block as Fig. 1, exposed to ribonuclease before staining. The basophilic bodies have disappeared. The few dots that remain are granules of hemosiderin.

FIG. 3. Adrenal from same case as Fig. 1, treated with veronal buffer before staining. The cells contain many basophilic bodies.

FIG. 4. Section of adrenal from same block as Fig. 3, exposed to ribonuclease before staining. The basophilic bodies have disappeared.

FIG. 5. Adrenal from case of widespread tuberculosis with perforation of ulcer of rectum followed by pyogenic peri-rectal abscess (Autopsy No. 17013). The section was treated with veronal buffer before staining. The basophilic bodies have a predominantly peripheral localization in the cells.

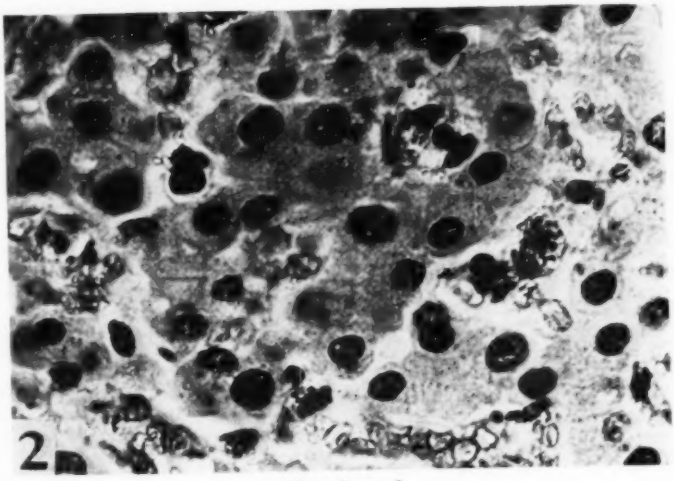
FIG. 6. Section of adrenal from same block as Fig. 5, exposed to ribonuclease before staining. The basophilic bodies have disappeared.

FIG. 7. Section of adrenal from same block as Fig. 3, treated with lanthanum chloride before exposure to ribonuclease. The lanthanum salt has protected the basophilic bodies from the action of the enzyme.

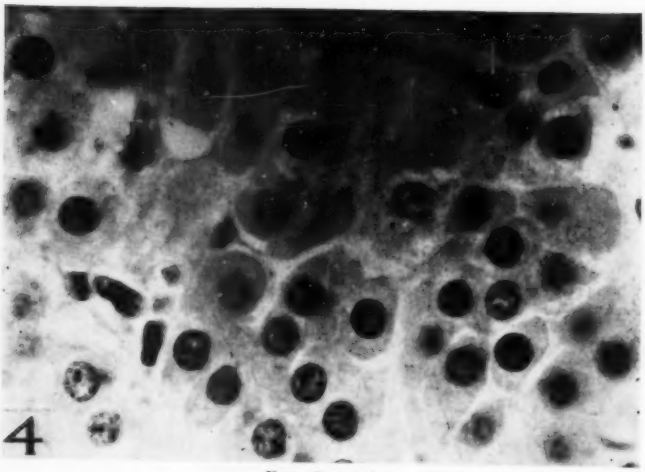
FIG. 8. Section of adrenal from same block as Fig. 7, treated with sodium chloride before exposure to ribonuclease. The basophilic bodies have been destroyed by the enzyme. Treatment with sodium chloride alone does not affect the bodies.

FIG. 9. Adrenal from case of reticular zone hyperplasia associated with precocious puberty (Surg. Path. No. 48–838), treated with veronal buffer before staining. There are many basophilic bodies in the hypertrophied reticular zone cells.

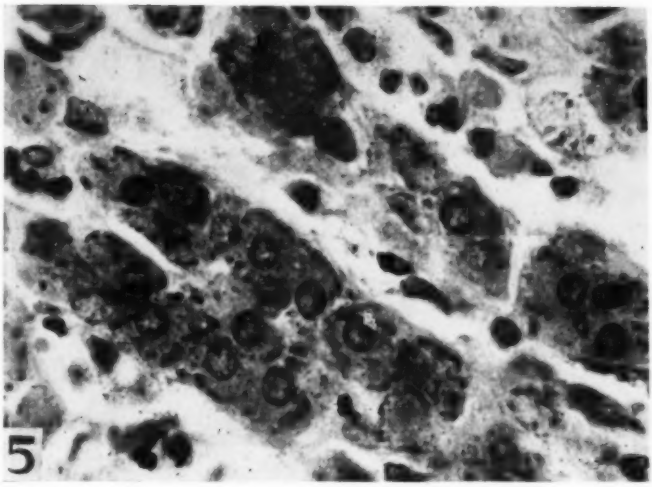
FIG. 10. Section of adrenal from same block as Fig. 9, treated with ribonuclease before staining. The basophilic bodies have disappeared.

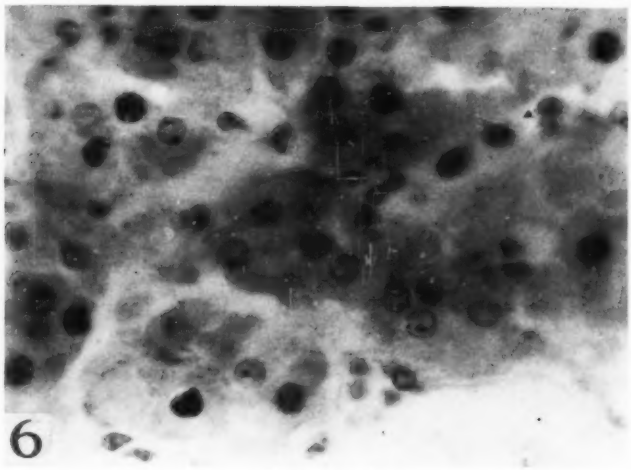


FIGS. 1 AND 2

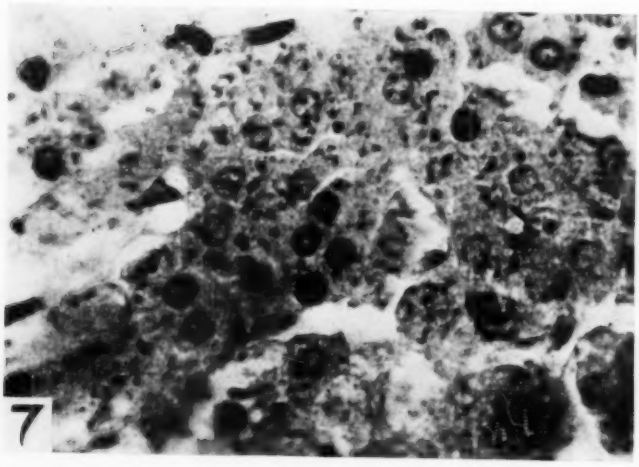


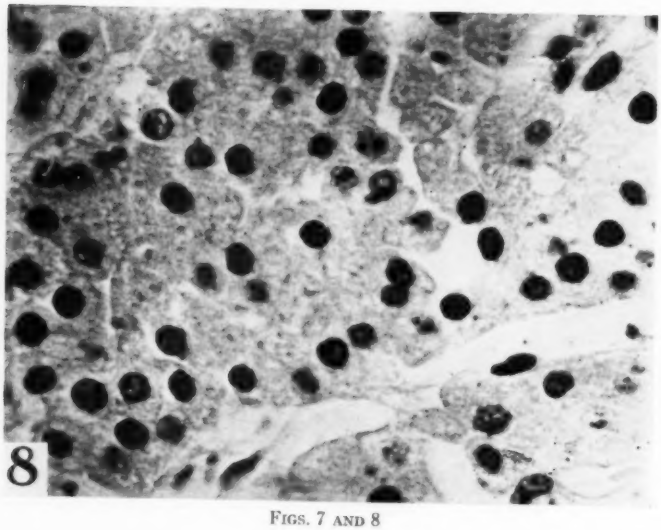
FIGS. 3 AND 4



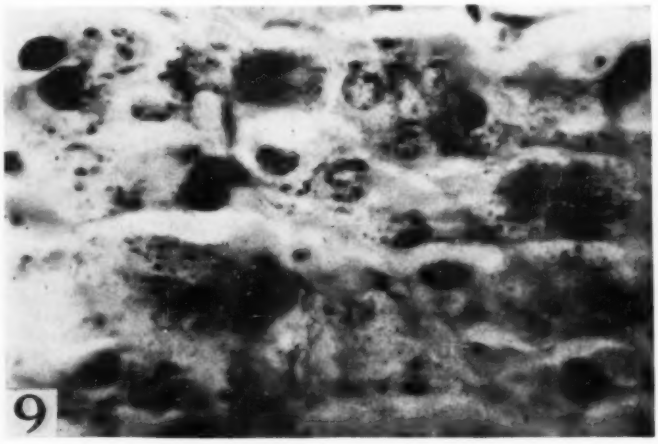


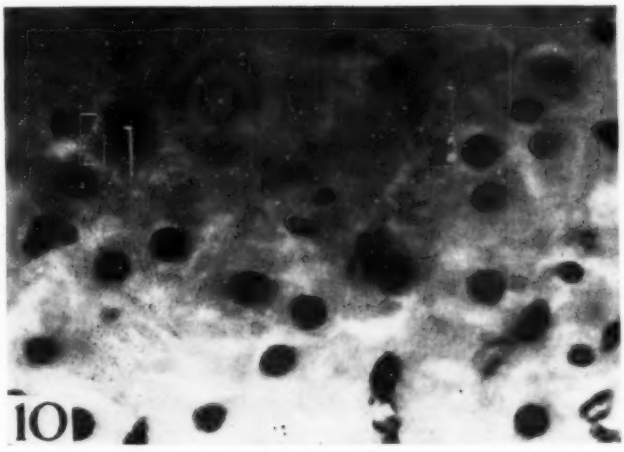
FIGS. 5 AND 6





FIGS. 7 AND 8





FIGS. 9 AND 10

ELECTROCORTICOGRAPHY

CURTIS MARSHALL AND A. EARL WALKER

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Received for publication August 15, 1949

INTRODUCTION

The recording of the electrical changes of the brain by electrodes placed directly upon the cerebral cortex has been termed electrocorticography.* Although such techniques have been used in animals since the beginning of electroencephalography, in man the procedure has only recently been carried out extensively. Its use is much more limited than electroencephalography, since, when the brain is exposed, the lesion is usually apparent. Yet electrocorticography has a definite place in neurological surgery, being of great value in anatomical and physiological studies of the cortex, in the localization of subcortical tumors and in the determination of epileptogenous foci.

TECHNIQUE OF ELECTROCORTICOGRAPHY

In early work (9, 14) the electrodes were merely laid on the cortex and not mounted on the skull. Surprisingly good records were obtained by this technique but the number of electrodes used was usually limited, only two being present on the cortex at once. For multiple recording some type of electrode holder, capable of being firmly clamped to the skull, is necessary. Several such types of assembly have been employed, perhaps the earliest being that used by Jasper (4). There are a number of models available at this time; all have similar principles but vary in design. The one used in this clinic (fig. 1) was devised by one of us (C.M.) and consists of a lucite plate in which 10–16 fine tubular electrodes are inserted, each upon a universal joint. The plate is attached to the skull by a clamp. Contact with the cortex is made by small

* Electrocorticography is to be distinguished from recording of scalp potentials (electroencephalography) and from the recording from subcortical structures with needle electrodes.

silver balls mounted on very light springs at the ends of wires floating in the tubular probes so that the pressure upon the cortex is always light. In some electrodes the cortical contact consists of a wick which is kept moist (4, 14). The electrodes should be freely movable so that they may be placed at any point over a wide area of the cortex.

Although a completely shielded room is desirable, electrocorticography may be carried out in the ordinary operating theatre with a good

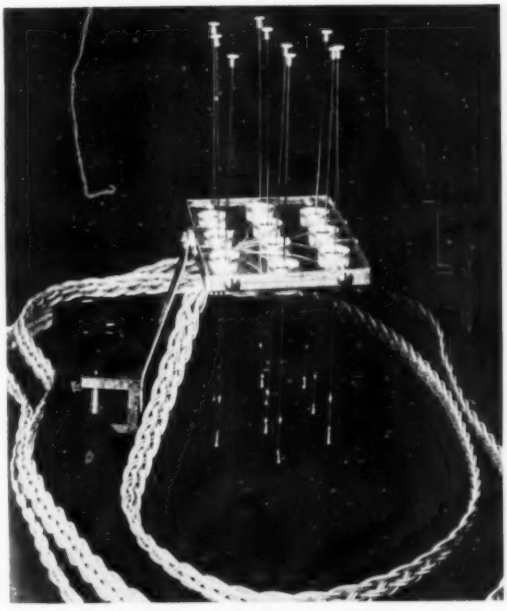

FIG. 1. THE ELECTRODE HOLDER

electroencephalograph, provided a few precautions are observed. The patient and operating table should be grounded to the electroencephalograph. Electrosurgical units, electric motors and sometimes the electric lights of the room must be disconnected at the wall plug. With these special arrangements usually a good record from the cortex may be obtained free of artifact. If spikes are interfering in all channels, an electric motor 30–50 feet away in an adjoining room may be at fault.

Occasionally such artifacts are in the line voltage and only with difficulty eliminated.

NORMAL ELECTROCORTICOGRAM

The traces obtained from the exposed cerebral cortex differ from those obtained from the scalp in two main characteristics (11). First, the amplitude of the cortical activity is 3–4 times greater than when sampled through the calvarium and scalp. Second, rapid oscillations in electrical activity are not distorted in the electrocorticogram and hence appear as spikes, whereas in the electroencephalogram interference by activity from adjacent areas or distortion by the distributed capacity of the intervening tissues may blunt their peaks. The characteristic of the waves must be relied upon in the analysis of the electrocorticogram, for obviously, comparisons cannot be made with comparable areas of the opposite side of the head. Usually only a part of one or more lobes of the brain is exposed so that the actual sampling of cerebral activity is from a very limited field even when eight channels are used. Usually bipolar recording from the brain is found most practical (4, 7, 9, 10, 14, 15) for an "indifferent" point may not be in the surgical field or may introduce artifactual disturbances.

It has been said that the electrocorticogram is dependent upon the cytoarchitectural structure of the cortex and that different areas have typical frequencies and patterns (6). In electroencephalography, the occipital, posterior parietal and temporal regions have an alpha rhythm (8–11/sec.) and the frontal regions a beta rhythm (14–22/sec.). This is also true for electrocorticography in the absence of general anesthesia, but the frequencies and pattern often change (fig. 2), and except for a few generalities there cannot be said to be a specific pattern for any one area (9, 10). Although at times it may be possible to recognize an area by its rhythm, as a rule, under controlled conditions, such prediction is no better than chance.

Anesthesia plays a great rôle in the characteristics of the electrical activity (fig. 3). Our experience has been confined to three states of anesthesia, local infiltration of the scalp, general ether anesthesia and intravenous pentothal anesthesia. Ether anesthesia causes a marked slowing of the cortical frequencies (9) with some increase in amplitude (fig. 3 C). This is present in all regions of the brain. Such slow activity

obscures all normal alpha rhythm and seems to depress the general excitability of the cerebral cortex so that epileptic discharges are suppressed and rarely seen. Pentothal anesthesia, at least in the surgical planes, augments the frequency and amplitude from all cortical regions

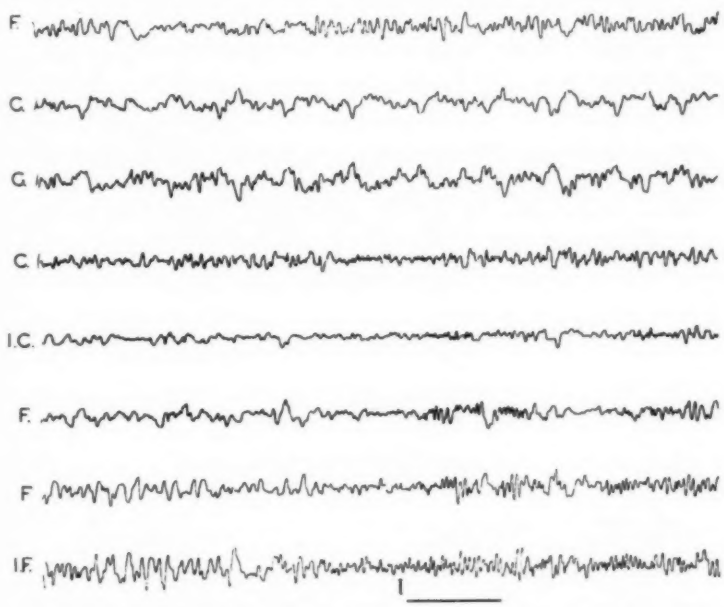

FIG. 2. Electrocorticogram of a patient operated upon under procaine infiltration of the scalp to show the marked changes in the spontaneous cortical activity. Note the transition from slow irregular activity in the frontal regions to regular 20 per second waves. The abbreviations are as follows: C, precentral cortex; F, frontal (prefrontal cortex); I.F. inferior frontal cortex. The horizontal line at the base of the record indicates an interval of one second, the vertical line a calibration of 200 microvolts.

(fig. 3 B). It does not seem to depress the cortical activity unduly, for epileptic discharges may still be seen. In general, the most accurate results can be obtained from electrocorticography when the craniotomy is done under local anesthesia, but at times satisfactory results may be obtained under general anesthesia. In epilepsy, when the subjective

responses of the patient are important, the bone flap may be reflected at a preliminary operation under general anesthesia and the cortical exploration carried out a week or ten days later with the scalp anesthetized with procaine.

Morphine, in the doses usually employed to allay anxiety, does not seem to depress cortical activity.

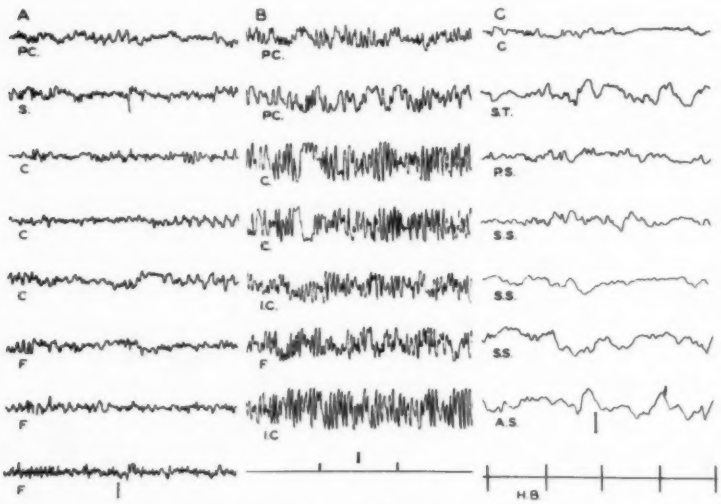

FIG. 3. Electrocorticograms to show the effect of anesthesia on the trace. A. Procaine used for anesthesia of scalp. B. Pentothal sodium intravenously. C. Ether. The abbreviations used are as follows: A.S., anterior Sylvian cortex; C, precentral cortex; F, frontal (prefrontal) cortex; H.B., heart beat; I.C., inferior cortex; P.C., postcentral cortex; P.S., posterior Sylvian cortex; S, Sylvian cortex; S.S., superior margin of Sylvian fissure; S.T., superior temporal gyrus.

The vertical lines at the base of the traces indicate a calibration of 200 microvolts; the horizontal line at the bottom of the second trace indicates intervals of one second.

Electrocorticography is subject to two influences not encountered in electroencephalography, which may produce artifacts in the traces—namely, brain pulsations due to respiration and cardiac action. On the other hand, muscle artifacts rarely are encountered in electrocorticography. The spiky waves produced by vascular pulsations may be recognized by their regularity and time relationships to an EKG put

in one channel of the electroencephalograph. Replacement of the electrodes to one side of the pulsating point will often eliminate the spike. Artifacts due to respiratory movement are slow, regular waves synchronous with respiration and can usually be eliminated by readjustment of the electrode.

ANATOMICAL AND PHYSIOLOGICAL STUDIES

Electrocorticography has been used little for anatomical studies in man. Yet it may prove to be a valuable tool as it has in the analysis of the fiber tracts in lower primates. The projection to or from cortical

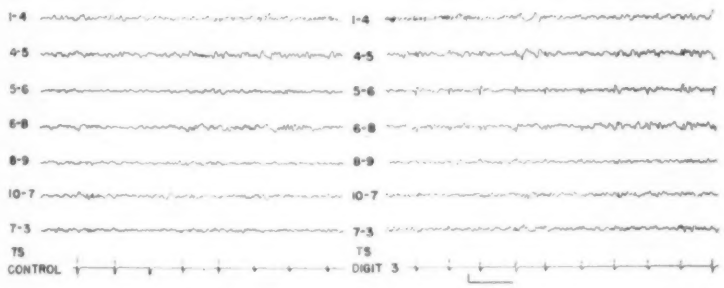

FIG. 4. Electrocorticograms showing the cortical potentials evoked by tapping the contralateral hand. The electrodes were placed along the postcentral convolution. Each tap is recorded on the lowest line. The control at the left shows the results of tapping the observer's hand which is holding the patient's fingers. The record at the right shows the response in the third and to a lesser degree in the fourth channel of tapping the patient's third finger.

neurones may be mapped out by the technique of evoked potentials. With the assistance of Dr. Clinton N. Woolsey, we have demonstrated the feasibility of utilizing in man the technique of cortical potentials evoked by peripheral cutaneous stimulation. This technique, as illustrated in Fig. 4, may be carried out with the patient under local anesthesia or under general ether anesthesia but not satisfactorily under pentothal anesthesia due to the amplitude of the cortical activity masking the evoked potential. In the primary sensory area, a diphasic response usually is seen. Somewhat more complex responses are obtained in the second somatosensory area along the superior margin of the posterior Sylvian region.

The study of fiber pathways, using strychnine neuronography has not proved very valuable in man. The strychnine spiking is rarely well developed in man and usually may be obtained only from a small area about the site of the application of the drug. Rarely does it give rise to spiking in areas farther than a few millimeters from this locus. A number of other analeptic drugs have been placed upon the human cortex with similar disappointing results. Metrazol, which fires the macaque cortex for 15 to 20 minutes is practically ineffective in evoking spike potentials in the human cortex unless an epileptic focus is present. Picrotoxin, if injected intracortically, may induce spike potentials but the technique has not been used to any great extent. It would seem that the present methods of neuronography in man are unsatisfactory. Perhaps some modifications will give more fruitful results.

Physiological observations of human cortical function are being made at the present time by an analysis of the cortical responses along the lines that Bishop and others have used in lower animals. The subcortical spontaneous epileptic spike may be used to study the cortical response in man (3). Other physiological studies have confirmed for man the applicability of phenomena previously shown in lower animals such as cortical extinction following a convulsive seizure.

LOCALIZATION OF TUMORS

Foerster and Altenburger (2) in 1935 showed that a tumor exposed at operation has no electrical activity but that compressed or infiltrated cortex at the margin of the neoplasm has abnormal activity characterized by slow waves. The preoperative localization of brain tumors on the basis of such delta activity is of great value to the neurological surgeon, but once a bone flap has been made the tumor is usually visible. Obviously electrocorticography is of little value in such lesions, but at times it may aid in the localization of a subcortical lesion (14, 15, 16). Slow waves interfering with normal frequencies usually indicate an underlying lesion (Fig. 5). Such slow waves may have a frequency of a half to two per second and an amplitude of 100 to 200 microvolts. A series arrangement of bipolar recording electrodes will enable a precise localization by determination of out of phase activity. This technique is applicable both to tumors of the cerebral hemispheres and cerebellum (Fig. 5A and 5B). Our limited experience with electrocortico-

graphy in deep-lying tumors of the midline or 3rd ventricle suggests that it is of little value.

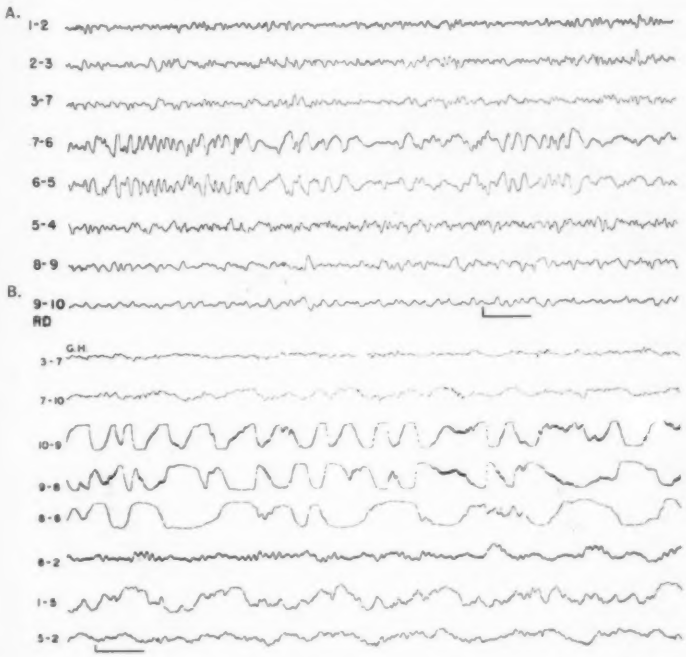

FIG. 5. A. Traces made from the cerebral cortex exposed under local anesthesia. A subcortical metastatic carcinoma was found under electrode 6.

B. Electrocorticograms of the cerebellum. Electrodes 1, 2 and 3 were over the right cerebellar hemisphere, 5, 6 and 7 were about the vermis and 8, 9 and 10 were on the left cerebellar hemisphere. A large cystic astrocytoma was found in the left cerebellar hemisphere.

LOCALIZATION OF EPILEPTIC FOCI

According to our present conceptions, focal epilepsy is due to abnormal discharge usually of pathologically altered neurones in an area of atrophic or degenerated cortex adjacent to a gross lesion. By electrocorticography in the conscious patient this epileptic focus may be determined by (1) focal spontaneous spiking, (2) induction of the aura by

electrical stimulation, (3) production of a long lasting after-discharge or (4) the initiation of spiking by local or systemic administration of metrazol.

Focal spontaneous spiking. In epileptics, the spontaneous electrical activity of the cerebral cortex has no constant pattern. The predom-

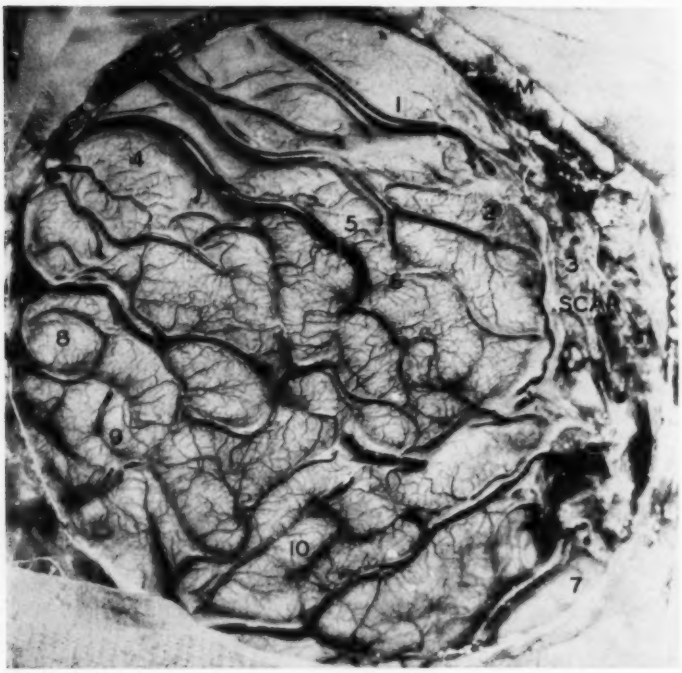

FIG. 6. Photograph of the exposed brain of a patient suffering from posttraumatic epilepsy. The scar and initial electrode setting are shown.

inant frequency varies from approximately 1 to 26 per second, but usually falling in the 8 to 11 per second range. About a scar, slow wave foci are consistently found (8) but are considered to be indicative of brain damage and not of epileptic activity. From the cortex adjacent to a scar, focal spontaneous electrical alterations, such as isolated spikes, bursts of spikes or spiky waves are present in approximately

one half of the posttraumatic epileptics examined. These abnormalities are usually confined to one gyrus and are present in an area of only a few square centimeters. Such spontaneous abnormalities are rarely associated with any clinical phenomena either subjective or objective.

The spike activity takes the form of individual monophasic or more often diphasic spikes, bursts of spikes or runs of spiky waves. This con-

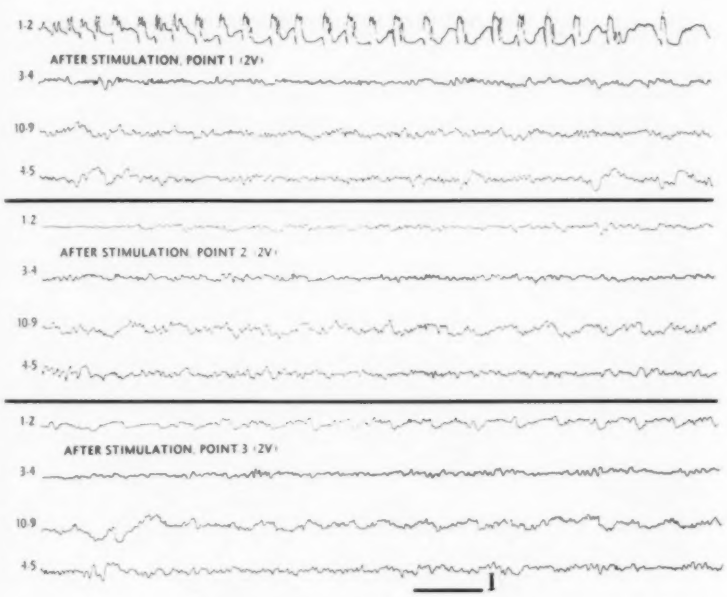

FIG. 7. Electrocorticograms following stimulation of points 1 to 3 as indicated. The remainder of the points stimulated seriatim at 1 minute intervals gave no prolonged after-discharge.

tinuous activity is usually confined to a relatively small area of the cortex and may change from moment to moment. Yet it was found in one patient to be present for many minutes.

Induction of the aura by electrical stimulation of the exposed cortex. The hyperirritable characteristic of the focus is confirmed by electrical stimulation. Ordinarily, representative points of the exposed cerebral cortex are stimulated by a sine wave current of 1.5 to 2.5 volts for 5

seconds. The electrical activity of the cortex and the motor and sensory responses are recorded. If no change is noted in the electroencephalogram after one minute another point is stimulated and so on until the entire cortex is mapped. The motor cortex can usually be located in this manner without provoking a convulsive seizure. A more accurate mapping of the motor responses may then be carried out. Stimulation

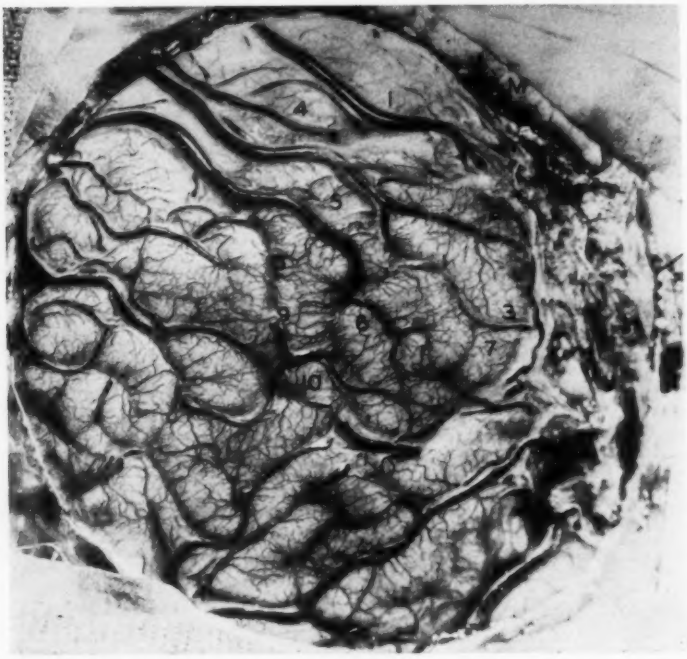

FIG. 8. The electrodes were grouped about the suspected epileptic area as shown

of the spiking area usually induces the patient's aura with or without a clinical or electrocorticographic seizure.

Production of a long lasting after-discharge. Occasionally an after-discharge occurs for a few seconds at the point of stimulation. Such a short lasting response up to 10 seconds is considered physiological. However, when the area spontaneously firing is stimulated with a cur-

rent too weak to elicit a response from normal cortex, a long lasting after-discharge usually occurs. If spontaneous spiking is not present,

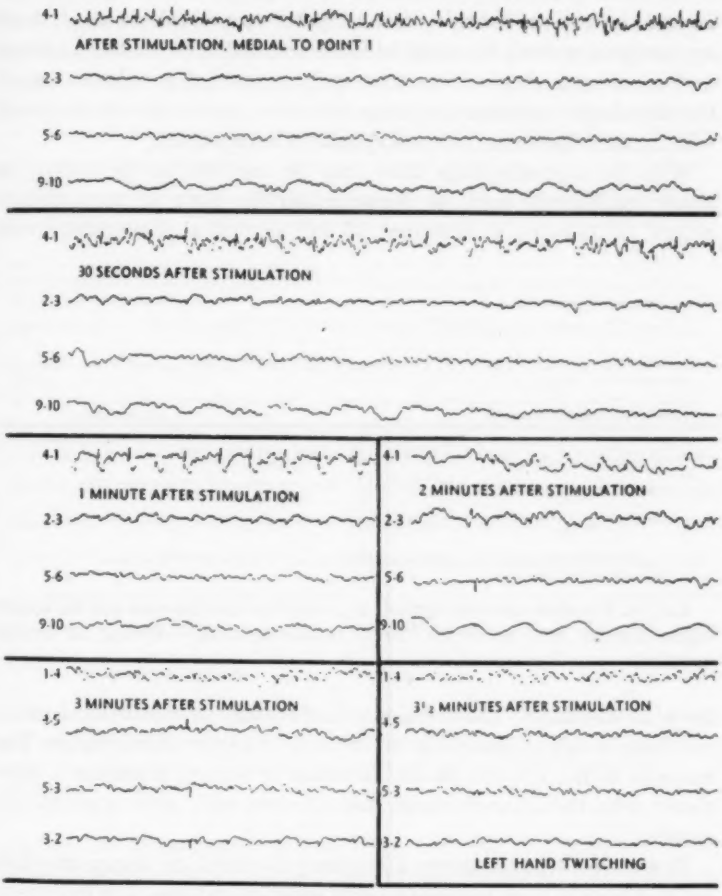

FIG. 9. Electrocorticograms at intervals after stimulation of point 1 showing the focal after-discharge.

generally an area of cortex, adjacent to the scar in posttraumatic epilepsy can be found which, when stimulated, causes a long lasting

spiky discharge. In one case this epileptic discharge lasted as long as 28 minutes. During this time the cortical activity of surrounding areas of the brain remained normal. During the period of focal epileptic discharge the patient thought he was quite normal and to all outward appearances seemed his usual self. He responded to simple questions and carried out simple commands and arithmetical problems. Only if the stimulation and after-discharge are in the motor areas or associated with an aura does consciousness appear to be impaired.

With the after-discharge there may or may not be the sensory or motor phenomena such as characterized the aura of the patient's attack. Of 33 cases, so activated, 13 had no clinical phenomenon with

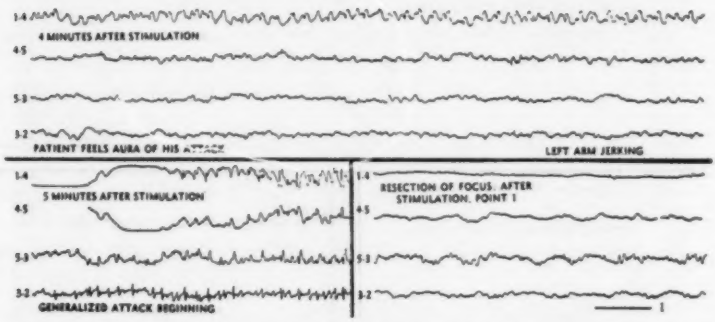
4 MINUTES AFTER STIMULATION
PATIENT FEELS AURA OF HIS ATTACK
LEFT ARM JERKING
5 MINUTES AFTER STIMULATION
RESECTION OF FOCUS. AFTER STIMULATION. POINT 1
GENERALIZED ATTACK BEGINNING
1

FIG. 10. The after-discharge spreads as the patient feels the aura and the seizure begins. The last trace shows the absence of after-discharge following the cortical resection.

the after-discharge. The remainder had sensory or motor focal manifestations which progressed in seven cases to a generalized seizure. The majority of the 13 cases having no motor or sensory phenomena associated with the after-discharge had no aura with their spontaneous attacks.

The after-discharge shows a tendency to build up during the first ten seconds and then assumes a fairly constant pattern. The frequency of the spiky waves decrease as the amplitude increases during the buildup. This is also true of the ending of the after-discharge. The pattern after the initial state may assume almost any form, but usually the hypersynchrony can be classified into one of five types: (1) spikes,

(2) saw tooth phenomena, (3) spike and dome forms, (4) phasic waves and (5) dicrotic waves and spikes. These forms may be continuous or paroxysmal, and/or regular or random.

Initiation of spiking by local or systemic administration of metrazol. In the early explorations, when spontaneous cortical spiking was not evident, small amounts of metrazol were injected intravenously to

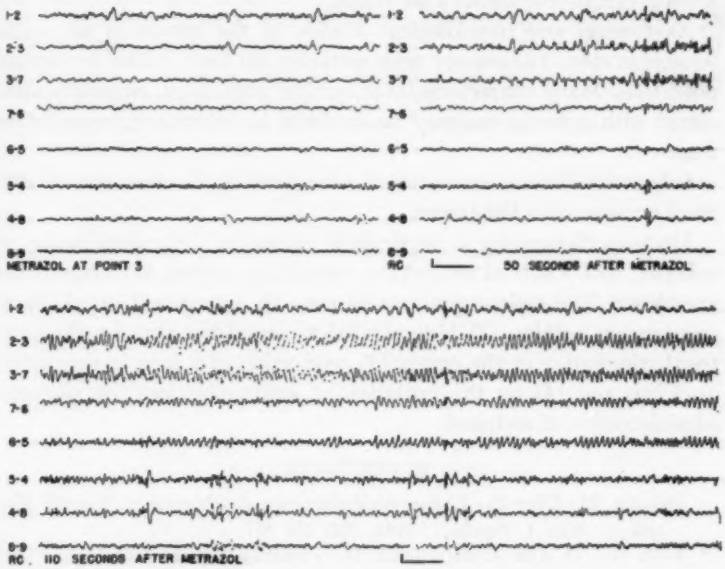

FIG. 11. Electrocorticograms showing the activating effect of a small amount of metrazol on a cotton pledget placed on an epileptogenic focus. In spite of the marked spiky activity the patient had no symptoms or signs of neurological dysfunction.

initiate the abnormal cortical activity. Our fear of the induction of a generalized seizure led us to abandon this technique. We discovered later that the foci in experimental epilepsy are hypersensitive to the local application of metrazol. In man we find that the intracortical injection of 0.1 cc of the usual metrazol solution produces no electrocorticographic changes in normal brains. But in epileptic foci even this small amount will initiate self-propagated discharges. In some cases

this technique gives highly satisfactory results but in other cases the areas adjacent to the real epileptic focus appear to be as hypersensitive to metrazol as the focus so that a clear-cut localization is not obtained.

SUMMARY

Electrocorticography may be carried out in the ordinary operating room if certain precautions are taken.

Anatomical and physiological studies of the cerebrum are quite feasible in man. The sensory representation has been studied by evoked potentials. Many characteristics of cortical physiology, especially associated with convulsions, may be explored by electrocorticography in man.

Subcortical brain tumors may be localized by the presence of slow wave activity over the tumor.

Electrocorticography is particularly valuable in the localization of epileptic foci. Cortical exploration should be carried out under local anesthesia. The epileptogenic focus may be determined by (1) focal spontaneous spiking (2) induction of a convulsive aura by the electrical stimulation of the cortex (3) production of a long lasting afterdischarge and (4) by the initiation of spiking by local or systemic administration of metrazol.

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STUDIES ON THE CHEMICAL DIFFERENTIATION OF DEVELOPING CARTILAGE AND BONE

I. General Method. Alkaline Phosphatase Activity*

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Received for publication August 18, 1949

In recent years much information has been accumulated on chemical changes which accompany growth and differentiation of the organism and its constituent tissues. To carry out such studies it is necessary to employ animals at various stages of development in order that progressive periods of morphological differentiation can be correlated with quantitative chemical determinations. Developing cartilage and bone, however, are unique among tissues in that all stages of their development may be found in a single specimen whether it be removed at an appropriate time in utero, at birth, or thereafter during the stage of active growth. Such tissues are therefore eminently suited for correlating the morphological alterations with the chemical changes which take place during growth and differentiation. In certain instances, of course, qualitative histochemical reactions may be employed and are useful (1). Such techniques are sometimes open to criticism, however; we have therefore turned to more quantitative techniques.

The present paper, which is the first of a series on the biochemistry of developing cartilage and bone, will describe the general method and its application to the study of one enzyme, alkaline phosphatase.

GENERAL METHOD

Two requirements must be met before utilizing the method to be described below. First, the specimen must be large enough to furnish sufficient material for chemical analysis. Second, the entire line of ossification must be in as level a plane as possible. In the specimens of cartilage and bone which have been studied the ribs from man and dog fulfill these two criteria. Long bones cannot be used since the diaphyseal portion of the cartilage is concave.

* Aided by a grant from Mead Johnson and Company.

The method consists in making horizontal slices of varying thickness through the cartilage in a plane parallel to the costochondral junction. One half of each slice is used for chemical study; the opposite half is prepared for histological examination.

Slices are cut on a Spencer Automatic Clinical Microtome (No. 888) whose knife clamp has been removed and replaced by a horizontal plate to which is fixed a razor blade (Durham-Duplex) (Fig. 1). An object clamp (Spencer No. 885) replaces the carbon dioxide freezing

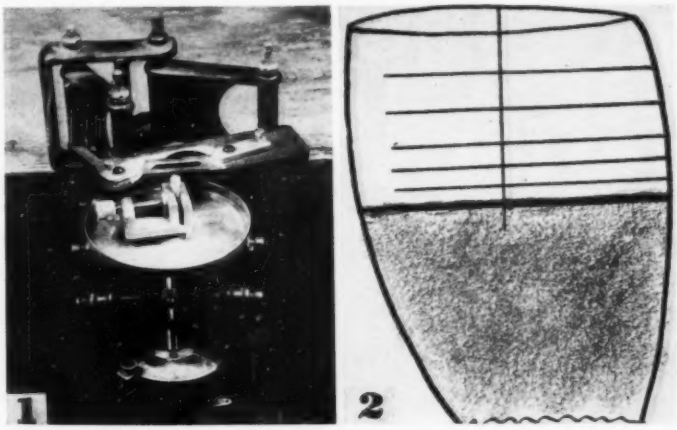

FIG. 1. Microtome assembly showing plate which is used to hold the razor blade in an horizontal plane.

FIG. 2. Schematic representation of slicing technique. Horizontal cuts are made three quarters or more through the cartilage. The final vertical slice frees the segments on the right for chemical analysis while those on the left are held together in one piece by the unsliced cartilage at the extreme left.

stage. The rib is clamped by the shaft in a vertical position with the line of ossification in as horizontal a plane as possible. This may be facilitated by transillumination of the region. The cartilage is cleaned of any adhering muscle or fat. The razor blade is then brought level with the line of ossification and, depending on the desired thickness, the stage is lowered by the vertical feed screw for a distance corresponding to the thickness of and number of slices desired. While the cartilage is steadied by manual counterpressure the blade is brought completely across the cartilage. The vertical feed screw is then turned and the

required number of slices of known thickness are made three quarters through the cartilage and on down to and including the cartilage shaft junction. When these transverse cuts have been completed, a mid-longitudinal slice is made from the free end of the cartilage down into the shaft (Fig. 2). This frees each slice nearest the blade; the fellow

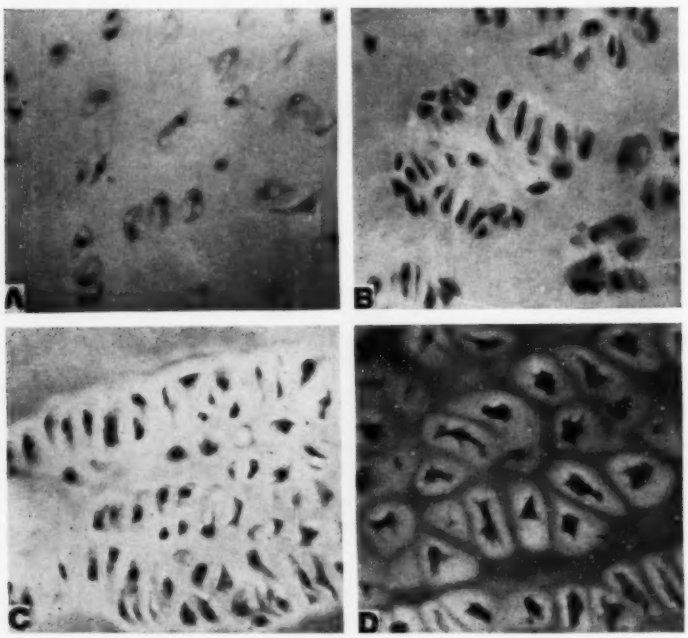

FIG. 3. Cartilage cells from various regions of differentiation; A, most undifferentiated; D, hypertrophic, most fully differentiated cells. See text for further explanation.

of each of these is held to the others by the remaining quarter which has not been sliced. The free halves are placed serially in a Petri dish and weighed on a torsion balance either immediately or after drying at 105°C. The entire hemisection of partially sliced cartilage is fixed in any desired solution and prepared for histological examination in the usual way, thus providing a series of segments separated by the incisions made by the razor blade.

Regions of the cartilage have been graded A, B, C and D depending on their degree of maturation. The cells in each of these 4 regions are shown in Figure 3. Region A is composed of the smallest, most undifferentiated cells. In region B the cells have become larger and are found in pairs; the cells in this region are undergoing division. In region C the cells are larger and have arranged themselves into rows. Region D consists of hypertrophic cells just above the invading blood vessels. In the present paper and those which will follow the slices which are studied will not necessarily consist entirely of each of these regions. There naturally is overlapping and the grading which is given is an average.

METHOD FOR PHOSPHATASE ACTIVITY

Slices are placed immediately without weighing in small serological tubes containing .5% beta glycerol phosphate solution buffered with sodium barbital at pH 10.9. They are shaken for 2 hours in a water bath maintained at 37°C. After the slices have been removed, 10% trichloracetic acid is added and the phosphorus content of an aliquot determined by the method of Fiske and Subbarow (2). The slices are dried at 100°C and weighed on a torsion balance. The alkaline phosphatase activity is expressed as milligrams phosphorus liberated in an hour per gram of dried tissue.

In the determination of periosteal phosphatase activity the pleura is removed from the inner surface of the rib and small segments of periosteum are stripped from the underlying cortex. These are treated in the same way as are the cartilage slices described above.

For the histological demonstration of alkaline phosphatase activity the technique of Gomori (3) has been applied to undecalcified sections, using 2 preparations: one incubated with glycerol phosphate and both then stained with silver nitrate.

RESULTS

In Table I is shown phosphatase activity at varying levels of differentiation (rated A to D) in the costal cartilage of the dog. There is slight yet definite activity in the zone of undifferentiated cells; as the region of hypertrophic cells is reached, a sudden increase in enzyme activity occurs. Quantitative values of periosteal phosphatase activity are likewise shown in Table I.

TABLE I

Correlation Between Cartilage Cell Differentiation and P-ase Activity in the Dog*

NUMBER	P-ASE ACTIVITY* VERSUS DIFFERENTIATION OF CARTILAGE CELLS						P-ASE ACTIVITY† IN PERIOSTEUM
	Slice 1	2	3	4	5	6	
1	1.1(A)	2.7(A)	3.8(A,B)	8.0(B,C)	20.5(C,D)	33.7(D,S†)	9.4
2	.5(A)	.5(A)	.5(A)	2.0(A,B)	7.8(C)	21.3(D)	6.1
3	.5(A)	.5(A)	.5(A)	.8(A)	2.6(B,C)	13.0(C,D)	6.5
4	.8(A)	.8(A)	.8(A)	.9(A)	2.5(B,C)	6.4(C,D)	4.0
5	.2(A)	.2(A)	.7(A)	1.5(B)	3.0(B,C)	3.8(C)	2.9
6	.2(A)	.2(A)	.4(A)	.7(B)	3.6(C)	8.1(D)	4.8
7	.2(A)	.2(A)	.2(A)	.3(A)	1.8(B,C)	3.9(C,D)	2.0
8	.3(A)	.3(A)	.5(A)	.5(A,B)	3.0(B,C)	8.7(D)	3.2
9	.2(A)	.3(A)	.3(A)	.4(A)	4.0(B,C)	4.2(C,D)	2.0

* Expressed as Mg. P liberated by 1 gram dry cartilage or periosteum per hour.
† S—Shaft of rib.

TABLE II

Correlation Between Cartilage Cell Differentiation and P-ase Activity in Children*

NUMBER	AGE	DIAGNOSIS	P-ASE ACTIVITY* VERSUS DIFFERENTIATION OF CARTILAGE CELLS						P-ASE ACTIVITY* PERIOSTEUM
			Slice						
			1	2	3	4	5	6	
669	3 mo.	Bronchiectasis	1.0(A)	1.0(A)	1.0(A)	2.0(B)	12.6(B,C)	24.2(C,D)	10.2
648	3 mo.	Pneumonia	1.1(A)	1.2(A)	1.4(A)	3.0(B)	10.0(C)	14.6(D)	2.7
653	3 mo.	Cardiac hypertrophy	.4(A)	.6(A)	.9(A)	5.1(B)	6.7(C)	8.8(D)	3.2
649	6 mo.	Cong. Mal. Heart	.5(A)	.5(A)	.5(A)	1.9(B)	7.4(C)	10.1(D)	5.3
702	6 mo.	Obstruction Trachea	.5(A)	.5(A)	.5(A)	1.5(A,B)	3.1(B,C)	9.6(C,D)	9.4
712	9 mo.	Hydrocephalus	.6(A)	.8(A)	.6(A)	.8(A,B)	2.9(B,C)	4.9(C,D)	2.3
727	15 mo.	Cong. Mal. Heart	.4(A)	.4(A)	.4(A)	.4(A)	.6(B)	11.5(C,D)	2.8
765	2 yr.	Cong. Mal. Heart	.3(A)	.3(A)	.4(A)	.4(A)	1.2(B)	7.0(C,D)	2.8
671	3 yr	Cong. Mal. Heart	.4(A)	.5(A)	.8(A)	.7(A)	1.5(A,B)	9.2(C,D)	5.6
674	3 yr.	Hydrocephalus	1.3(A)	1.4(A)	1.3(A)	2.1(B)	2.4(B,C)	18.0(D,S†)	—
725	3 yr.	Neuroblastoma	.6(A)	.6(A)	.6(A)	.6(A)	2.3(B,C)	4.7(C,D)	4.8
748	3 yr.	Cong. Mal. Heart	.2(A)	.2(A)	.3(A)	.3(A)	.6(B)	4.9(C,D)	5.7
719	4 yr.	Meningitis	.4(A)	.4(A)	.4(A)	.4(A)	1.4(B,C)	6.3(D)	3.0
763	6 yr.	Cong. Mal. Heart	.3(A)	.3(A)	.3(A)	.3(A)	.3(A,B)	1.7(C)	3.0
718	7 yr.	Poliomyelitis	.4(A)	.4(A)	.4(A)	.4(A)	3.5(B,C)	4.8(C,D)	2.4
755	7 yr.	Cong. Mal. Heart	.4(A)	.5(A)	.5(A)	1.5(B,C)	4.9(C,D)	—	1.9
665	12 yr.	Hydrocephalus	.1(A)	.2(A)	1.2(B)	1.5(B,C)	2.3(C,D)	—	2.0
704	14 yr.	Rheumatic Ht. Dis.	.4(A)	.4(A)	.4(A)	1.7(B)	3.6(C,D)	4.3(D,S†)	8.6

* Expressed as Mg. P liberated by 1 gram dry cartilage or periosteum per hour.
† S—Shaft of rib.

In Table II are summarized values of phosphatase activity at varying levels of cartilage cell differentiation and in the periosteum of a group of children dying of a variety of causes. These data indicate the variability in quantitative results which are encountered. Age as well

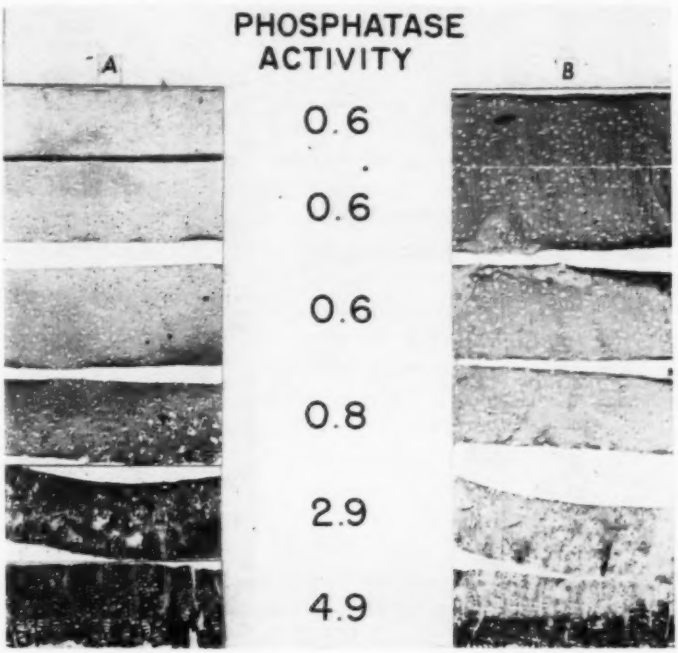

FIG. 4. Quantitative values of phosphatase activity in the costal cartilage of a child (712) compared with lower power microscopic sections from the same regions. A and B are serial sections; section B was incubated with glycerol phosphate for 2 hours. Both were then stained with silver nitrate. Note darker zone in B. Areas of lime salt deposition naturally stain black in both.

as the state of nutrition appear to have an effect on the values which have been obtained. Because of this variability, observations such as these would seem to have little significance in comparative studies of the cartilage of children. They, of course, do confirm in a quantitative way the high concentration of phosphatase at the costochrondral

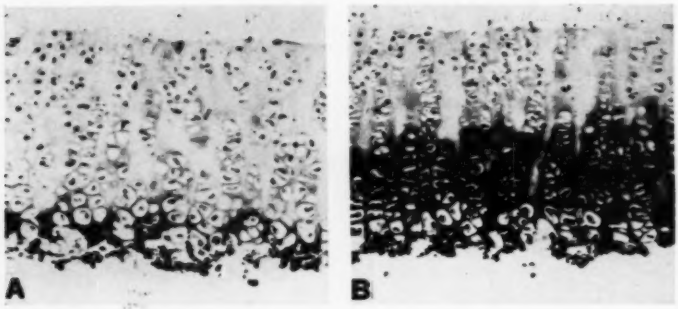

FIG. 5. Higher power view of lowermost slices shown in Figure 4, A and B. Difference in dark staining material indicates presence of phosphatase activity.

TABLE III

Phosphatase Activity of Adult Human Periosteum

NUMBER	AGE	SEX	P-ASE ACTIVITY*	CAUSE OF DEATH
700	59	M	1.0	Bronchogenic Carcinoma
703	54	M	1.0	Carcinoma Prostate
706	57	F	1.1	Subdural Hematoma
707	24	F	1.1	Nodular Cirrhosis
708	70	F	.8	Generalized Arteriosclerosis
711	43	F	.7	Carcinoma Breast
715	65	F	.9	Cong. Heart Disease
716	35	F	1.3	Meningioma
717	30	F	1.0	Acute Pancreatitis
721	58	M	.6	Glioma
723	45	F	3.5	Sarcoma Involving Vertebrae
724	69	M	3.5	Bronchogenic Carcinoma
725	60	F	1.3	Carcinoma Cervix
730	60	F	.5	Carcinoma Cervix
746	42	M	2.2	Vascular Nephritis
759	63	M	.6	Myeloid Leukemia
761	67	F	.4	Adhesive Pericarditis
766	20	F	.9	Thrombosis Portal Vein
771	54	F	.6	Peritonitis

* Expressed as Mg. P Released Per Hour Per Gram Dried Tissue.

junction. Values of periosteal activity in this group of children are also shown in Table II. Figure 4 illustrates the correlation between morphological differentiation, histochemical demonstration of phosphatase ac-

tivity and quantitative chemical determination of enzyme activity. Such a comparison reveals that there is chemical evidence of enzyme activity in the zone of undifferentiated cells though such may not be demonstrated by the histochemical method which has been used. Figure 5 shows the distribution of phosphatase activity by histochemical technique.

Values of phosphatase activity from the periosteum of a series of adults dying of a variety of causes are shown in Table III. With certain exceptions these values tend to be fairly uniform and group themselves about an average of .8 mg. P released per gram of dry tissue per hour. The exceptions are cases No. 723, No. 724 and No. 746. The first (No. 723) was a peculiar tumor of the mediastinum with involvement of the vertebrae. The serum calcium and phosphorus values were 11.3 and 5.1 mg. percent respectively. Case No. 724 was a squamous cell carcinoma with local invasion; the cause for the elevation of phosphatase activity is not clear. Case No. 746 died of chronic renal insufficiency on a vascular basis; serum calcium and phosphorus values were 7.8 and 6.6 mg. percent respectively. The increased periosteal phosphatase activity in this case does not come as a surprise in view of the histological changes which may be encountered in the skeletal tissues of individuals with renal disease (4).

DISCUSSION

Little discussion is necessary regarding the general method which has been employed in this study. It is, of course, a variant of the technique which Linderström-Lang (5) has utilized so profitably. We are more fortunate, however, that, as a result of the architecture of cartilage, single slices rather than alternate ones may be used for both chemical and histological study.

Since the demonstration of alkaline phosphatase in skeletal tissues by Robison (6) in 1923, an extensive literature dealing with the rôle of this enzyme in the mechanism of calcification has accumulated. It seems unnecessary to review at this time the details of this controversial subject, recently so well covered by Moog (7) and Roche (8).

The observations which we have presented on the quantitative increase in phosphatase activity in direct relation to differentiation and maturation of cartilage cells confirm and amplify the data which have

been reported on cartilage and bone of man (9) and the rat (10) and of explanted avian bone (11). In addition they provide confirmation and place the qualitative observations obtained by histochemical techniques (12, 13) on a firm quantitative basis.

Alkaline phosphatase activity in periosteum was observed histochemically by Robison (14) early in his studies on this enzyme. As a matter of fact to Robison rather than to later workers should go the credit for the histochemical demonstration of phosphatase activity. Other more elegant procedures on tissue sections have, of course, confirmed the presence of phosphatase activity in periosteum. However to our knowledge no attention has been given to the quantitative measurement of periosteal enzyme activity. We have felt that this tissue provides the purest culture of osteoblastic cells obtainable; there is little reason not to assume that strips of periosteum will reflect the activity of osteoblasts in general. The reservation must be made, of course, for local increase in activity about fractures, tumor metastases, etc. We are at present carrying out a series of observations on periosteal phosphatase activity in disease in order to amplify the material presented in Table III. Such data, when augumented by studies on serum calcium, phosphorus concentrations and phosphatase activity should prove most interesting.

SUMMARY

A method is described with which to correlate the morphological and chemical changes which take place in the normally differentiating costal cartilage of the dog and man

Quantitative values of alkaline phosphatase activity in costal cartilage of these two species have been correlated with the degree of differentiation of the cartilage cells.

Quantitative data on alkaline phosphatase activity in the periosteum of normal dogs and children and adults coming to autopsy are presented.

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ON CREATINURIA IN MAN. THE ROLES OF THE RENAL TUBULE AND OF MUSCLE MASS[1, 2]

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Received for publication September 8, 1949

Urinary excretion of abnormal quantities of creatine is a recognised concomitant of a variety of pathological states. Since the ultimate composition of urine is regulated by the kidney, it is useful to consider the causes of creatinuria in terms of renal function.

Modern concepts define two factors which determine the urinary excretion of substances not secreted by the renal tubules: (1) the rate of glomerular filtration and (2) the rate of tubular reabsorption. In these terms creatinuria occurs whenever the creatine tubular load (mg. creatine filtered by the glomeruli per minute) exceeds the creatine reabsorption rate (mg. creatine reabsorbed by the renal tubules per minute). This disproportion might result from increased tubular load, decreased reabsorption rate or a combination of both.

Tubular load is the mathematical product of the glomerular filtration rate and the concentration of creatine in plasma; its augmentation would depend on an increase in one or both of these factors. Since significant acceleration of the glomerular filtration rate has not been observed in man in circumstances associated with creatinuria, excessive creatine tubular loads must in most instances, therefore, reflect elevated plasma creatine concentrations. Factors which might increase the quantity of circulating creatine are presented in Table 1 (I, B); essentially, these are the conditions which Hunter suggested might produce creatinuria (19).

Factors which determine tubular reabsorptive rate for creatine are little understood. The sole example of modification of this function has been demonstrated in the dog infused with glycine (32).

[1] Work done under a contract between the Office of Naval Research, U. S. Navy Department, and the Johns Hopkins University.

[2] Presented in part at the annual meeting of The American Society for Clinical Investigation, May 3, 1948.

Classical examples of creatinuria in man occur in such diffuse disturbances of muscle as the muscular dystrophies. However, creatinuria is associated with a number of circumstances apparently unrelated to massive muscular disease. It was the main purpose of the study reported here to determine whether or not, in these latter circumstances, modification of tubular reabsorptive activity contributed to the appearance of creatinuria. Measurements of renal function with respect to creatine were performed, therefore, under the following conditions:

TABLE 1

Causes of Creatinuria

Expressed in Terms of Renal Function

	CLINICAL EXAMPLES
I. Increased tubular load of creatine	
A. Accelerated glomerular filtration rate...	None
B. Elevated concentration of serum creatine	
1. Exogenous source of creatine.....	Ingestion (31, 37)
2. Accelerated synthesis of creatine....	Ingestion of precursors (4) Methyltestosterone (39)
3. Extrusion of intracellular creatine...	Acute denervation atrophy (12) ? Starvation (3) ? Acute immobilisation (7)
4. Inadequate disposition of creatine...	Muscle lack (this paper)
II. Decreased tubular reabsorptive rate of creatine	
A. Competitive blocking of tubular mechanism.....	Hyperaminoacidemia (32)
B. Basis unknown.....	Desoxycorticosterone Thyroid substance Puerperium Cushing's syndrome (this paper)

(1) during the puerperium, (2) in thyroid disease, (a) during the early response to thyroid substance by hypothyroid subjects, and (b) in hyperthyroidism, (3) in Cushing's disease, (4) during prolonged administration of desoxycorticosterone acetate (DCA)[3] and (5) during administration of methyltestosterone (MT).[4]

[3] Percorten, generously supplied by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

[4] Generously supplied by Schering Corporation, Bloomfield, New Jersey.

A second possible basis for creatinuria is reduction in total muscle mass leading to inadequate disposition of creatine (Table 1: I, B, 4). Prior clinical examples of this type of creatinuria are in the main inconclusive because of the coexisting factor of progressive muscle wasting. As a first approximation of the role of muscle lack in spontaneous creatinuria, a study was made, therefore, of two patients with extensive loss of muscle mass due to obsolete anterior poliomyelitis.

I. CREATINURIA DUE TO DIMINISHED TUBULAR REABSORPTION

The role of the renal tubule was evaluated by simultaneous measurements of renal clearance of creatine and the glomerular filtration rate in certain circumstances associated with creatinuria.

The procedures for the determination of renal function were performed with the subjects in the post-absorptive basal state. Urine collections were obtained by catheterization of the patients and by voluntary voiding by the normal subjects; collection periods were approximately 20 minutes each. Adequate urine flows were insured by administering water perorally. A sample of venous blood was obtained once during each period. In one subject from whom samples of both arterial and venous blood were obtained, creatine concentrations were identical within the limit of error of the method. The concentration of the appropriate solute at the midpoint of the period was obtained by inspection of the curve formed by plotting the logarithm of the concentration of the solute in serum against time.[5] The glomerular filtration rate was determined by calculating the clearance of inulin, mannitol or of sodium thiosulfate from the expression $C = \frac{UV,}{P}$ where C is the glomerular filtration rate (ml./min.), U is the concentration of the

[5] Renal clearance of the endogenous chromogen giving the Jaffe reaction ("creatinine" clearance) is a function of the glomerular filtration rate. Since inulin clearances were determined on the basis of a falling serum concentration, while "creatinine" clearances were determined on the basis of a constant endogenous serum concentration, variations in urine flow would be expected to disturb the "creatinine" clearance: inulin clearance ratio if so-called renal delay time were a factor of practical import. However, in the observations reported here, the ratio of "creatinine" clearance to inulin clearance was constant and independent of urine flow during short-term measurements in a given subject. Renal delay time, therefore, has not been considered in calculation of clearances.

solute in urine (mg./ml.), P is its concentration in serum (mg./ml.), and V is the rate of urine flow (ml./min./1.73 m^2 body surface).

Clearances of inulin and of mannitol were determined after a single injection. Clearances of sodium thiosulfate were determined during a constant infusion. Chemical methods were those of Harrison for inulin (15), Smith for mannitol (35) and Newman for sodium thiosulfate (27).

Creatine clearances (C_C) were determined during rising serum concentrations before and after oral administration of 20 to 25 g. of creatine. Analyses for creatine and creatinine were performed according to the method of Peters (29).

Since there is analytical error in the measurement of the rate of excretion of small amounts of endogenous creatine during acute tests of renal function, these data were checked, as a first approximation, in the case of one subject (B. R) by expressing the creatine excretion rate, determined during an acute test, as a 24-hour output and comparing it with the known output of creatine during the day preceding the test. When it is considered that renal clearances were performed under basal conditions, these values, presented in Table 2, are in virtual agreement and support the validity of the method.

From raw data were derived the following:

(1) The ratio, creatine clearance: glomerular filtration rate, C_C/C. This represents the proportion of filtered creatine which is excreted in the urine; that is, it is equal to the creatine excretion rate divided by the tubular load of creatine (*vide infra*).

(2) The proportion of filtered creatine which is reabsorbed; $(1 - C_C/C)$.

(3) The tubular load of creatine, or the rate at which creatine is filtered through the glomerulus: $P_C \times C$, where P_C is the serum concentration of creatine (mg./ml.).

(4) The creatine excretion rate: $U_C \times V$, where U_C is the concentration of creatine in the urine (mg./ml.).

(5) The creatine reabsorption rate, or the difference between the tubular load and excretion rate: $T_C = (P_C \times C) - (U_C \times V)$, where T_C is the creatine reabsorption rate. The same value is, of course, obtained as the product of the tubular load and the proportion of creatine which is reabsorbed: $T_C = (P_C \times C)(1 - C_C/C)$. Since crea-

tine reabsorption rate, particularly at endogenous loads, was calculated as the relatively small difference between two much larger terms, reliance cannot be placed on its absolute value. For this reason, tubular activity was assessed as a function of C_C/C (Table 4, column 8 and Fig. 1).

When increments in tubular load of creatine no longer result in an increased T_C, the rate of maximum tubular reabsorption of creatine, (Tm_C), has been reached.

Implicit in the calculation of the renal clearance of creatine is the assumption that all of the creatine in serum is free to be filtered through the glomeruli. If this assumption is incorrect, alterations in diffusibility of creatine may alter the apparent tubular reabsorption. The essential validity of the assumption, however, has been established by the demonstration that creatine was distributed uniformly between serum and certain extracellular fluids, as indicated in Table 3, and by the fact that the creatine concentration in anerobic ultrafiltrates of serum, separated by the technic of Lavietes (20), was found to equal that in serum water.

In 79 urine-collection periods the endogenous "creatinine" clearance was compared to the inulin clearance. The ratio of these clearances was independent of the rate of urine flow which varied over twenty-fold and was constant in a given subject during any series of urine-collection periods. Sixty-five of the 79 clearance ratios (82 per cent) differed from their mean by no more than 10 per cent. This constancy is considered evidence for the accuracy of urine collections. It is apparent that, by the nature of the experimental procedure, inaccurate urine collections would produce divergent errors in the determination of creatine clearance (performed during *rising* serum concentration) and the glomerular filtration rate (performed during *falling* serum concentration of inulin or mannitol) and render the ratio C_C/C virtually meaningless.

Tubular reabsorption of creatine in the normal. Renal clearances of creatine were measured on four occasions in two healthy young women. The results appear in Table 6 and, in part, in Figure 1B. In agreement with the observations of Tierney and Peters (37), no creatine appeared in the urine when the serum concentration of creatine was less than 0.5 mg./100 ml., and creatinuria always occurred when the serum

concentration exceeded that value. Despite the achievement of tubular loads which were 25 to 90 times greater than the endogenous load, no Tm_C was demonstrated; i.e., the tubular reabsorptive mechanism was not saturated. The highest creatine reabsorption rate observed in each of the four experiments varied from 9.1 to 23.0 mg./min./1.73 m^2. The ratio, C_C/C, tended to become constant at high creatine loads, approximately associated with concentration of serum creatine in excess of 10 mg./100 ml. In ten observations performed at these high loads, the ratio C_C/C varied from 0.55 to 0.81, with a mean of 0.68. These data are in agreement with those of Pitts (31) who compared the creatine clearance to the xylose clearance in a normal woman. When the xylose clearance is corrected to give a measurement of the filtration rate (xylose clearance = 0.75 × inulin clearance), it can be calculated from Pitts' data that his female subject had, at high tubular loads of creatine, a tubular reabsorption rate of 6 to 11 mg./min. (uncorrected for body surface), and a ratio C_C/C of 0.73.

Effect of desoxycorticosterone acetate. B. R., Unit No. 393446, a 58-year-old male with myotonic dystrophy, a non-toxic nodular goiter, bilateral testicular atrophy, and gynecomastia, received 180 mg. of DCA in oil intramuscularly, divided in daily doses for 17 days. During the first 11 days of DCA administration he retained sodium and water, gained weight, and had a less positive potassium balance. During the last six days of DCA administration he was in negative sodium and potassium balance, displayed mild polydipsia and polyuria and lost weight. Details of the electrolyte and water metabolism in this subject have been reported elsewhere (41).

Simultaneous clearances of mannitol and of creatine were determined before DCA administration and on the seventeenth day of DCA administration. The results appear in table 4. Prior to the exhibition of DCA, the ratio C_C/C rose with increasing serum concentration toward a plateau of approximately 0.8, indicating that as much as 20 per cent of filtered creatine was reabsorbed. Tm_C was approximately 3 mg./min./1.73 m^2 body surface. DCA induced complete inhibition of creatine reabsorption under the conditions noted. The ratio C_C/C approximated unity at all serum concentrations of creatine and Tm_C was zero (Figure 1A).

Thyroid disease and effect of thyroid substance in hypothyroidism.

Renal clearances were measured before therapy and ten to 14 days after institution of thyroid therapy in three patients with hypothyroidism.

H. G., Unit No. 372322, a 37-year-old housewife, had myxedema due to Hashimoto's struma, demonstrated by biopsy of the thyroid. Her BMR was −16 per cent of normal and her serum cholesterol concentration was 252 mg. per cent. Two weeks after introduction of treatment with desiccated thyroid, 64 mg. daily, the patient experienced

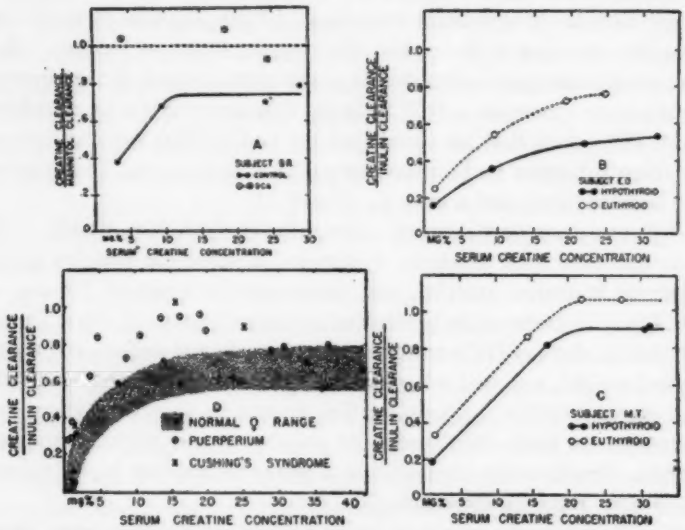

FIG. 1. Relation of creatine clearance to glomerular filtration rate at various levels of serum creatine. On the ordinate a value of 0 indicates complete reabsorption of creatine, and 1.0 indicates no reabsorption.

a diuresis, her weight fell from 145 lbs. to 134 lbs. and dryness and puffiness of her face began to disappear. BMR rose to −4 and, ultimately, to +9 per cent of normal. Serum cholesterol concentration fell to 165 mg. per cent.

E. D., Unit No. 366610, a 53-year-old housewife, had idiopathic hypothyroidism manifested by increasing fatigue, weight gain, dryness of skin and hair, coarse timbre of voice, dyspnea, an enlarged tongue and pitting peripheral edema. Her BMR was −27 per cent of normal

and her serum cholesterol concentration was 328 mg. per cent. With administration of thyroid substance, 96 mg. daily, she improved. Her BMR rose to +2 per cent of normal and the serum cholesterol concentration fell to 215 mg. per cent.

M. T., Unit No. 443502, a 22-year-old domestic, had hypothyroidism manifested by inability to concentrate, increasing fatigue and gain in weight. Her BMR was −25 per cent of normal and her serum choles-

TABLE 2

Comparison of Actual and Calculated 24-Hour Excretions of Creatine

Subject B. R.

PERIOD	G. CREATINE/24 HRS./1.73 m^2 b.s.	
	Calculated	Actual
Control	0.084	0.098
DCA	0	0.015
MT (45th day)	1.253	1.365

TABLE 3

The Equilibrium Concentrations of Creatine in Serum Water and Extracellular Water (8 patients)

CREATINE MG. PER CENT		SOURCE OF FLUID
Serum Water	Extracellular Fluid Water	
0.0	0.1	Pleural
0.3	0.2	Peritoneal
0.3	0.3	Edema
0.6	0.5	Edema
0.6	0.4	Peritoneal
0.7	0.7	Pleural
0.8	0.8	Edema
1.3	1.3	Peritoneal

terol concentration was 366 mg. per cent. With administration of thyroid substance, 64 mg. daily, the BMR rose to +3 per cent of normal and the serum cholesterol concentration fell to 290 mg. per cent.

Exhibition of thyroid substance diminished tubular reabsorptive activity; the ratio C_C/C increased in each instance. (Table 4, Figure 1B and C.)

In three male subjects with hyperthyroidism tubular reabsorptive

TABLE 4

Modification of the Renal Clearance of Creatine

SUBJECT AND BODY SURFACE	REMARKS	GLOMERULAR FILTRATION RATE (ml./min.) C	CREATINE				CLEARANCE RATIO CREATINE: FILTRATION RATE Cc/C
			Serum I(mg. %) Pc × 100	Filtered (mg./min.) Pc × C	Excreted (mg./min.) Uc × V	Reabsorbed (mg./min.) (Pc × C) − (Uc × V)	
1	2	3	4	5	6	7	8
M. J.							
1.70 m²	Normal female	118 (I)	1.35	1.59	0.09	1.50	0.05
		127	7.40	9.40	5.50	3.90	0.59
		140	24.50	34.30	20.90	13.40	0.61
		110	33.80	37.20	26.30	10.90	0.71
1.69 m²		116.3 (I)	0.93	1.08	0.32	0.75	0.30
		111.9	16.20	18.10	11.00	7.10	0.59
		111.7	31.00	34.70	27.70	7.00	0.80
		104.6	43.50	45.40	29.80	15.60	0.67
1.69 m²		115.4 (I)	0.48	0.55	0	0.55	0
		110.3	13.70	15.10	10.80	4.30	0.71
		115.9	28.80	33.40	26.10	7.30	0.77
		132.9	37.00	48.80	39.70	9.10	0.81
D. F.							
1.58 m²	Normal female	152.6 (I)	1.07	1.63	0.17	1.46	0.11
		153.3	23.50	36.00	22.20	13.80	0.62
		151.9	36.00	54.60	31.60	23.00	0.55
K. Z.							
1.78 m²	Normal male	131.6 (I)	0.31	0.41	0	0.41	0
		114.3	3.65	4.17	2.14	2.03	0.51
		112.8	10.50	11.85	8.41	3.44	0.71
		119.2	17.50	20.85	13.88	6.97	0.67
J. L.							
1.96 m²	Normal male	120.6 (I)	0.46	0.55	0.15	0.40	0.28
		140.3	6.20	8.70	4.98	3.72	0.57
		120.4	11.14	13.41	10.73	2.68	0.80
		124.3	15.60	19.40	14.35	5.05	0.74

All rates are referred to 1.73 m² body surface. Creatine values are presented in hundredths of mg. as determined, although the accuracy of the analytic method is limited to four parts in one hundred.

(I) Inulin clearance.

(M) Mannitol clearance.

(T) Sodium thiosulfate clearance.

TABLE 4—*Continued*

SUBJECT AND BODY SURFACE	REMARKS	GLOMERULAR FILTRATION RATE (ml./min.) C	CREATINE				CLEARANCE RATIO CREATINE: FILTRATION RATE Cc/C
			Serum (mg.%) Pc × 100	Filtered (mg./min.) Pc × C	Excreted (mg./min.) Uc × V	Reabsorbed (mg./min.) (Pc × C) − (Uc × V)	
1	2	3	4	5	6	7	8
H. G.							
1.61 m²	Myxedema	78.9*(T)	0.35	0.28	0	0.28	0
	BMR −16	78.9	4.10	3.23	0.82	2.41	0.25
		78.9	14.00	11.03	9.05	1.98	0.82
		78.9	22.50	17.75	14.22	3.53	0.80
1.60 m²	During thyroid	64.5*(T)	0.96	0.62	0.45	0.17	0.73
	treatment	64.5	9.00	5.80	3.87	1.93	0.67
	BMR −4	64.5	26.00	16.75	15.84	0.91	0.94
		64.5	38.00	24.50	22.90	1.60	0.93
M. T.							
1.68 m²	Myxedema	117.8(I)	0.95	1.12	0.21	0.91	0.19
	BMR −25	110.8	17.10	18.93	15.45	3.48	0.82
		106.9	30.50	32.60	29.58	3.02	0.91
		114.0	31.00	35.35	32.62	2.73	0.92
1.69 m²	During thyroid	116.1(I)	1.38	1.60	0.54	1.06	0.34
	treatment	125.5	14.30	17.93	15.58	2.35	0.87
	BMR +3	108.3	21.80	23.62	25.07	−1.45	1.06
		114.6	27.30	31.30	33.20	−1.90	1.06
E. C.							
1.67 m²	Myxedema	76.0(I)	1.02	0.78	0.12	0.66	0.16
	BMR −27	68.5	9.20	6.30	2.26	4.04	0.36
		69.5	22.00	15.28	7.57	7.71	0.49
		104.0	32.00	33.28	17.58	15.70	0.53
1.68 m²	During thyroid	115.2(I)	1.29	1.49	0.37	1.12	0.25
	treatment	85.4	9.50	8.12	4.39	4.73	0.54
	BMR −7	90.1	19.50	17.59	12.58	5.01	0.72
		92.6	24.90	23.10	17.50	5.60	0.76

* Owing to unpredictable but sometimes large changes in creatine excretion with alterations in sodium excretion (42), the measurements of creatine clearance were made during four consecutive periods and then the GFR was measured in the following four periods by infusing $Na_2S_2O_3$. The average of these GFR determinations was applied to the preceding periods. Justification for this extrapolation is found in the observation that, during the first experiment on subject H. G., renal clearance of endogenous creatinine was not altered by infusion of $Na_2S_2O_3$. Thus, during the three clearance periods immediately preceding $Na_2S_2O_3$ infusion, creatinine clearance averaged 86.6 (90.3, 81.6 and 88.1) and the ratio of creatinine clearance to the average thiosulfate clearance was 1.14, 1.03 and 1.12 respectively. During the three clearance periods in which $Na_2S_2O_3$ was infused, the creatinine clearance averaged 81.3 (76.2, 82.7 and 84.9) and the ratios of creatinine clearance to the corresponding thiosulfate clearance were 0.96, 1.05 and 1.07, respectively.

TABLE 4—*Concluded*

SUBJECT AND BODY SURFACE	REMARKS	GLOMERULAR FILTRATION RATE (ml./min.) C	CREATINE				CLEARANCE RATIO CREATINE: FILTRATION RATE Cc/C
			Serum mg.(% Pc × 100	Filtered (mg./min.) Pc × C	Excreted (mg./min.) Uc × V	Reabsorbed (mg./min.) (Pc × C) − (Uc × V)	
1	2	3	4	5	6	7	8
B. R.							
1.66 m²	Myotonic dystrophy, untreated	48.7(M)	3.19†	1.55	0.60	0.95	0.39
		56.3	9.50	5.35	3.63	1.72	0.68
		54.9	24.00	13.18	9.17	4.01	0.70
		44.7	28.70	12.83	9.91	2.92	0.77
1.66 m²	During DCA	68.6(M)	9.10†	6.25	6.52	−0.27	1.04
		83.9	18.50	15.53	17.00	−1.47	1.10
		50.2	24.30	12.21	11.26	0.95	0.92
1.66 m²	During methyltestosterone (45 days)	67.5(I)	4.35	2.94	0.91	2.04	0.31
		68.3	5.00	3.42	1.35	2.07	0.40
		73.8	13.00	9.60	8.76	0.84	0.91
		56.8	24.60	13.97	10.32	3.65	0.74
1.65 m²	During methyltestosterone (50 days)	71.3(I)	4.40	3.14	1.09	2.05	0.35
		84.1	13.00	10.93	6.81	4.12	0.62
		55.9	25.50	14.27	13.59	0.68	0.95
		60.2	44.50	26.79	19.05	7.74	0.71
D. L.							
1.69 m²	Cushing's disease	97.3(I)	1.24	1.21	0.43	0.78	0.36
		96.0	12.40	11.90	7.27	4.63	0.61
		86.5	15.60	13.50	13.97	−0.47	1.04
		86.8	25.40	22.05	19.80	2.25	0.90
M. S.							
1.37 m²	Post-partum 5th day	160.0(I)	0.75	1.20	0.34	0.86	0.28
		170.0	3.36	5.72	3.61	2.11	0.63
		179.0	14.00	25.05	18.10	6.95	0.72
		166.0	19.90	33.02	29.05	3.97	0.88
H. D.	Post-partum 5th day	113.8(I)	1.03	1.17	0.44	0.73	0.38
1.81 m²		110.0	8.60	9.46	6.92	2.54	0.73
		116.8	16.60	19.40	18.43	0.97	0.95
		117.8	19.00	22.39	21.50	0.89	0.96
S. C.							
1.65 m²	Post-partum 4th day	165.6(I)	0.96	1.59	0.46	1.13	0.29
		167.8	4.70	7.90	6.65	1.25	0.84
		162.6	10.60	17.23	14.58	2.65	0.85
		166.8	13.60	22.65	21.40	1.25	0.94

† These periods do not represent endogenous levels but levels following the ingestion of creatine. In all other experiments the first period represents measurements made just before ingestion of creatine.

activity was normal with respect to creatine. Renal clearance determinations, repeated in two subjects when they had become euthyroid, revealed no change in the ratio C_C/C. In each subject during his hyperthyroid phase endogenous creatine concentration in serum was greater than normal for the male, in agreement with the observations of Tierney and Peters (37), and was reduced with treatment. Creatinuria in hyperthyroidism, then, may be the result of increased tubular load, an end result of several possible pre-renal changes (Table 1, IB).

Puerperal creatinuria. It has long been known that a profound creatinuria accompanies the first week or two of the puerperium. At one time it was held that puerperal creatinuria was the result of release of creatine from the involuting myometrium (34), but it has been calculated that more creatine is excreted in several days postpartum than is contained in the entire gravid uterus (2). Renal clearances were measured in three subjects on the fourth or fifth day postpartum. The three subjects, Unit Nos. 437362, 402467, 409667, all had had normal, full-term pregnancies with uneventful labors and uncomplicated post-partum periods. All were lactating. Data on renal performance appear in table 4 and figure 1D, together with data on a group of normal subjects. The ratio C_C/C during the puerperium well exceeded the normal. Furthermore, since the concentration of endogenous creatine in the serum was essentially normal while the ratio C_C/C was elevated, puerperal creatinuria appears to be the result of reduced tubular reabsorption of creatine. It will be noted that in two of the three subjects GFR was greater than the normal range, creating an increased tubular load. However, this increase would not produce creatinuria of the degree displayed by these subjects if the efficiency of tubular reabsorption of creatine were normal. At maximum tubular loads, creatine reabsorption rate was only a fraction of normal.

Lack of effect of methyltestosterone. MT, 50 mg. daily, was administered orally to the myotonic subject B. R. for 30 days, during which time creatine excretion rose from zero to more than 1400 mg. a day. During the peak of creatinuria simultaneous clearances of inulin and creatine were measured on two occasions.

MT was without effect on tubular mechanisms for creatine reabsorption (Table 4), but was accompanied by a rise in serum creatine to a concentration of more than 4 mg. per cent, at which level creatinuria

of the same order occurs in the normal subject who has received a large dose of creatine by mouth (Figure 1D).

Cushing's disease. D. L., Unit No. 479544, a 26-year-old singer, complained of deepening of her voice, thinning of her scalp hair, acne of face and arms, increase in body hair, gain in weight with especial roundness of her face. Her appearance was typical of Cushing's disease. She had abdominal striae, hirsutism and enlargement of the clitoris. Her blood pressure was 165 mm. Hg systolic and 105 mm. Hg diastolic. There was slight osteoporosis of the lumbar spine. Fasting blood glucose concentration and glucose tolerance were normal. Serum sodium concentration was as high as 152.5 mEq./1. She excreted increased amounts of 17-ketosteroids (32–39 mg./day), but not of 11-oxysteroids (0.4 mg./day). There was eosinopenia (0 — 55 per cu. mm.).

Data on renal performance appear in table 4. The ratio C_C/C was greater than normal, and at maximum tubular loads the creatine reabsorption rate was less than half of the lowest normal value.

II. CREATINURIA ASSOCIATED WITH REDUCED MUSCLE MASS

Although it has been proposed for about 30 years (19) that a reduction in available muscle storage space forces creatine to overflow into the urine, the clinical material which has been studied does not afford a clear demonstration of this possible cause of creatinuria. Relevant reports have dealt with states in which muscle atrophy was progressive (23, 24). In these states it may be considered that dissolution of muscle adds creatine to extracellular fluid and that a portion of this increment may appear in the urine. Determination of the creatine content of the urine, in such cases, does not permit a decision as to whether creatinuria results from extrusion of intracellular creatine or from inadequate disposition of newly-synthesised or ingested creatine. In order to isolate one of these factors from the other, there were selected for study of this problem two patients with extensive muscular atrophy due to obsolete anterior poliomyelitis, in whom the possibility of progressive repair or destruction of muscle was remote. The assumption was made that the remaining muscle cells retained normal function. The clinical state of these patients precluded the complete study of renal function which was planned. The fragmentary observations

which were made are recorded here, nevertheless, because they support an hypothesis concerning one mechanism responsible for certain instances of creatinuria.

L. R., Unit No. 398745, a 24-year-old white female, had been bedfast for eight years following an acute episode of anterior poliomyelitis in 1938. She weighed 60.8 kg. and her surface area was 1.60 m^2. Flaccid paralysis was complete in all extremities, thorax, trunk and neck. Breathing was entirely diaphragmatic. Although calculi were demonstrable roentgenographically in the right kidney, no evidence of impaired renal function was obtained. Arterial pressure was 110 mm. Hg systolic and 70 mm. Hg diastolic. On one occasion a few leucocytes appeared in the urine; on other occasions urinalysis was normal. At the end of two hours 45 per cent of an intramuscular dose of phenolsulphonphthalein was recovered in the urine. Blood non-protein nitrogen concentration was 20 mg. per cent. There was no evidence of abnormal catabolic activity; the patient had no recent weight loss, fever or leucocytosis, and her basal metabolic rate was −21 per cent of normal.

When preliminary analysis revealed creatinuria, nitrogen balance was assayed to determine whether or not the creatinuria in this bedfast patient might be similar to that reported to occur during acute immobilisation (7). The patient was placed on a constant diet calculated to contain 50 g. of protein, 58 g. of fat, 117 g. of carbohydrate and 1200 calories per day. Included in this diet were 50 g. of meat. Identical menus were offered each day. An aliquot representing one-half of one day's ration of each five-day batch of food was analysed for nitrogen by a macro Kjeldahl method (5). Pooled five-day stools were dried on a steam bath and analysed for nitrogen. Urine was collected daily under 10 ml. of 25 per cent acetic acid and analysed for nitrogen and guanidoacetic acid (8).

The results of these determinations appear in Table 5. There was a profound creatinuria and a marked reduction in creatinine excretion. The concentration of creatine in the serum was elevated to 1.6 mg. per cent. That this increase in tubular load of creatine was not the result of extrusion of creatine from the intracellular mass and was unlike the creatinuria observed in acute immobilisation is suggested by the presence of positive nitrogen balance on a moderate nitrogen in-

take (0.8 g. of protein per kg. body weight). With certain reservations to be discussed below, the normal excretion of guanidoacetic acid is considered to be compatible with a normal velocity of synthesis of creatine. A conservative estimate of tubular activity may be made by assuming that the glomerular filtration rate was normal and that the post-absorptive concentration of creatine in the serum prevailed throughout the day. The amount of creatine filtered, then was approximately 100 ml./min. × 0.016 mg./ml. × 1440 min./day = 2304 mg./day. Of this quantity, 714 mg. appeared in the urine on the day on which the serum was obtained. The proportion of creatine which was reabsorbed was, therefore, $\left(1 - \frac{714}{2304}\right) \times 100$, or 69 per cent, which

TABLE 5

Daily Excretion of Creatine Bodies and Nitrogen Balance in Obsolete Poliomyelitis

DAY	URINE			
	Nitrogen Balance	Guanidoacetic	Creatinine	Creatine
	g.	mg.	mg.	mg.
1–5	+1.59	48.5	216	925
6–10	+0.85	42.7	211	904
11–15	+1.48	54.4	226	893
16–20	+0.57	—	212	965

Subject L. R. Body weight, 60.8 kg. Nitrogen intake 8.1 to 8.3 g./day by analysis. All values are expressed as averages for 24 hours. Serum creatine concentration, 1.6 mg. per cent.

is a low normal tubular performance at a serum concentration of 1.6 mg. per cent.

At the completion of the balance periods, subject L. R. was placed on a creatine-poor diet and, after equilibrium to the new intake had occurred, a creatine tolerance test was performed by the technic of Thorn (36).

A second subject was observed similarly on a creatine-poor diet. This subject, B. A., Unit No. 240356, a 28-year-old white female, developed acute anterior poliomyelitis in 1941 with initial flaccid paralysis of the thorax and all four extremities. Following recovery from the acute phase, her activity was slightly less restricted than was that of the previous subject, and she regained about 60 per cent of normal function in the right upper extremity.

The data on urinary excretion of creatine and creatinine during creatine-poor intake appear in Table 6. Both subjects displayed pronounced creatinuria and a reduced excretion of creatinine. Creatine tolerance was severely impaired.

If the assumptions are correct, it would appear that mere loss of muscle mass is an adequate cause of creatinuria in obsolete anterior poliomyelitis, on the principle that creatine, synthesised at normal velocity and lacking adequate muscle space for its disposition, overflows into the urine. A plausible derivative is that creatinuria may be expected as a non-specific phenomenon wherever there is sufficient muscle wasting, provided that synthesis and reabsorption of creatine proceed at normal rates.

TABLE 6

Urinary Excretion of Creatine Bodies in Obsolete Anterior Poliomyelitis

	NORMAL (60 KG. B. W.) FEMALE	SUBJECT	
		L. R.	B. A.
Creatinine (mg./day)	840–1300	180	304
Creatine (mg./day)	0–60	714	588
Creatinine coefficient	14–22	3	5
Total creatinine coefficient	14–22	13.3	13.2
Creatine tolerance (per cent)	70	10	27

It is interesting to note that over a period of years no measurable adjustment in rate of creatine production was made in the face of diminished peripheral requirement for creatine.

DISCUSSION

Causes of creatinuria in terms of renal function have been listed in Table 1. Measurement of glomerular filtration rate (GFR) and of renal clearance of creatine will determine whether a given instance of creatinuria is the result of increased tubular load, decreased tubular reabsorption rate or both. If the creatinuria is associated with decreased reabsorption rate, presently available technics do not permit further differentiation. If creatinuria results from an increased tubular load some differentiation may be made between its two components: GFR and the serum concentration of creatine. It is unlikely that in spontaneous disease an increase in GFR occurs in sufficient mag-

nitude to cause significant creatinuria; in most instances increased tubular load will reflect elevated concentration of serum creatine.

Indirect assessments of *velocity of creatine synthesis* in man have been made by following the fate of isotopic creatine (18) and by measuring the urinary excretion of guanidoacetic acid (4). The isotopic technic is refined but not easily applied to clinical study at this time. With respect to guanidoacetic acid it remains to be demonstrated that renal tubular mechanisms do not modify its urinary excretion. Until this possibility is studied conclusions regarding creatine synthesis must be tentative.[6]

Extrusion of intracellular creatine might be expected to operate when a significant number of muscle fibers disrupt as, for example, during rapid atrophy in acute anterior poliomyelitis. In the presence of negative metabolic balances of nitrogen, phosphorus, potassium and sulfur, in ratios peculiar to their relative concentrations in muscle protoplasm, it may be surmised that creatine similarly escapes the cell. It is conceivable, too, that under certain circumstances creatine might escape from muscle cells without accompanying leakage of other cellular constituents. It is not possible presently to dissect these mechanisms free from other concurrent factors which may be operating to produce creatinuria. One of these factors is considered immediately below.

Inadequate disposition of creatine. It is reasonable to suppose, on the basis of the observations reported here on subjects with obsolete anterior poliomyelitis, that creatinuria occurs when reduction in muscle mass is great enough to render residual tissue insufficient for proper disposition of creatine. Essential to this supposition is the maintenance of normal velocity of synthesis and rate of tubular reabsorption. The assumptions used in the calculations above appear reasonable, but the observations do not preclude the possibility that a minor fraction of the creatine excreted resulted from slight diminution in tubular reabsorption.

It is possible that a minor reduction in muscle mass does not cause creatinuria. In such cases deposition of creatine may occur in liver, kidney, skin or remaining muscle since these tissues will absorb large

[6] Sims and Seldin have observed that tubular reabsorption of guanidoacetic acid diminishes as the tubular load of creatine increases (34a).

amounts of extra creatine in the rat (6). Such alternate deposition apparently may be limited in man. For example, in a patient who had undergone an amputation of the leg and half the pelvis for sarcoma we have observed an elevated serum creatine concentration and significant creatinuria three weeks post-operatively when the patient was afebrile, ambulatory and had exhibited no obvious loss of remaining muscle.

Speculations on the lack of creatinuria in myotonic dystrophy. There are some striking exceptions to the observation that creatinuria accompanies reduction in muscle mass: myotonic dystrophy (9), certain instances of hyperthyroidism (28) and, perhaps, hypoadrenalism (11, 40). In a subject with reduced muscle mass absence of creatinuria might result from increased tubular reabsorption, augmented metabolism of creatine by alternate pathways or decreased velocity of synthesis. These possibilities have been surveyed in the case of myotonic dystrophy.

Lack of creatinuria in myotonic dystrophy is not due to increased tubular reabsorption. In two patients with severe muscular wasting due to this disease the concentration of creatine in serum was normal (zero to 0.4 mg. per cent). Appropriate measurements of renal functions in one of these patients (B. R., Table 4) indicated that creatine was reabsorbed with normal efficiency. Two observations suggest that there is not significant metabolism of creatine along pathways alternate to its conversion to creatinine; (1) the pattern of rising concentration of creatine in the serum after an oral dose of creatine was normal and (2) a profound hypercreatinemia was achieved and maintained by administration of MT.

There remains the possibility that the velocity of creatine synthesis is decreased in myotonic dystrophy. It is concluded from the response to MT that the *capacity* to synthesise creatine is not defective. In the light of this observation it may be suspected that some undefined stimulus normally concerned with the full production of creatine is lacking in myotonic dystrophy.

Significance of tubular reabsorption of creatine. The renal tubules of normal adult man on a creatine-poor diet reabsorb some 560 mg. of creatine each day (assuming a serum creatine concentration of 0.003 mg./ml. and a GFR of 130 ml./min.). These tubules are capable,

however, of reabsorbing completely a load of some 1000 mg. of creatine per day (renal threshhold for creatine is approximately 0.005 mg./ml. (37), GFR is 130 ml./min. (10), and 1440 min./day: $0.005 \times 130 \times 1440 = 936$ mg./day or 0.65 mg./min./1.73 m^2 b.s.). Since creatinuria of the order of but 100 mg. per day is considered significant, a reduction of only 10 per cent of the capacity for reabsorption will result in notable creatinuria. Thus, when the rate of creatine reabsorption is depressed creatinuria may appear or increase. An example of such depression in the dog has been reported by Pitts (32) who effected a reduction in creatine reabsorption by infusing glycine, and concluded that α-amino acids competed for the same tubular transfer agency. In man, depression of creatine reabsorption has been observed during administration of DCA to patients with myotonic dystrophy, of thyroid substance to patients with hypothyroidism, in a patient with Cushing's syndrome, and spontaneously during the puerperium. In none of these conditions is there a rise in the serum concentration of α-amino acids sufficient to account for the creatinuria, if the phenomenon is comparable in man and dog. This does not exclude the possibility that the creatinuria accompanying diabetic acidosis, for example, results in part from the significant hyperaminoacidemia which occurs (21).

There is no readily apparent causal factor common to the various instances of depression of creatine reabsorption which have been described here. In the case of DCA, collateral evidence suggested that widespread reduction in general tubular reabsorptive activity had occurred (41). This was indicated partly by the mild diuresis which accompanied the creatinuria. Such mild diureses accompany the early period of therapy in the hypothyroid patient and the early puerperium. That water diuresis alone does not induce creatinuria has been established in this laboratory by producing wide variations in urine flow, both acutely and over 24-hour periods, without altering creatine excretion or reabsorption (42).

Data presented in this report indicate that when thyroid substance was exhibited to patients with hypothyroidism an early effect was decreased tubular reabsorption of creatine. Wilkins and Fleischmann (38) observed that, with respect to urinary excretion of creatine and creatinine, the effects of thyroid treatment of myxedematous patients

fell into two phases. During the first phase the output of creatinine was not changed greatly, but creatine excretion increased so that the total output of creatine and creatinine exceeded that of the hypothyroid state. In the second phase, the total output of creatine and creatinine was approximately equal to that of the hypothyroid state or perhaps slightly less. In this phase creatinine excretion was decreased and creatine excretion increased compared to the levels before treatment. Our observations were made during the first phase. It was at this time that the subjects studied by Wilkins and Fleischmann were largely in negative nitrogen balance. Synchrony between negative nitrogen balance and creatinuria is frequent. It occurs in starvation (3), during acute immobilisation (7), and during convalescence from acute illnesses (13). Wilkins and Fleischmann considered that in two of their subjects the nitrogen deficit was too slight to account for the increments in creatinuria in terms of dissolution of protoplasm. It is possible, therefore, that the parallel changes in nitrogen excretion and creatinuria are in some measure the common result of depression of renal tubular reabsorption. While the data at hand warrant no conclusion concerning the mechanism of creatinuria in starvation, immobilisation and convalescence, these states may be associated with the process called adaptation (33) which has been linked with adrenal cortical activity. That adrenal corticoids influence renal tubular reabsorption of creatine is suggested by the observations reported in the patient with Cushing's disease and in the case of administration of DCA. Whether or not the creatinuria accompanying the first few weeks of thyroid administration in myxedema and the creatinuria of the puerperium are examples of adaptation cannot be discerned from the evidence at hand. It may be recalled simply that during the early puerperium profound homeostatic adjustments are in full course in many systems.

Relationship of the creatinine coefficients to renal function. The constancy of the creatinine coefficient (mg. creatinine excreted in 24 hours/kg. body weight) has been emphasised repeatedly; a narrow range of values is found for normal men (20–26) and for normal women (14–22) (40). It has been held on reasonable grounds that, since the main source of creatinine is the creatine of muscle, the creatinine coefficient is a function of muscle mass. However, the observations reported here

on the modification of creatine excretion effected by the kidney suggested the necessity for further analysis of the renal factor in creatinine excretion. Endogenous creatinine is not reabsorbed actively by the renal tubule. Because tubular secretion of true endogenous creatinine is virtually insignificant in normal man, endogenous creatinine clearance may be an approximate measure of the glomerular filtration rate (26).[7] Thus, the amount of creatinine excreted is determined largely by the GFR and by the plasma concentration. A reduction in either of these factors might decrease the creatinine coefficient. With diminishing GFR, but with maintained muscle output of creatinine, the serum creatinine rises; e.g., renal insufficiency. Conversely, if renal function is maintained but muscle output is diminished serum creatinine falls. Examples of this latter situation were found in three patients with hyperthyroidism in whom the serum concentrations of true creatinine were low but then rose during treatment concurrently with a return of body weight and muscle bulk, without significant alteration of GFR. Thus, it would appear that in most cases an estimate of muscle mass derived from the value of the creatinine coefficient must presuppose a normal GFR; and, conversely, an estimate

[7] The concentration of true endogenous creatinine in serum may be approximated from the expression $P = \frac{UV}{C}$, when P is the serum concentration of true endogenous creatinine, UV is the rate of urinary excretion of creatinine (mg./min.) determined by the Jaffe reaction, and C is the GFR measured by inulin, mannitol or thiosulfate clearances. The assumptions inherent in this expression are dependent upon the observations that (*a*) true endogenous creatinine clearance is an approximate measure of GFR (1, 26) and (*b*) the preformed Jaffe chromogen in urine, in contrast to serum, is virtually all true creatinine (25). When P was calculated in this fashion in 84 clearance periods in man, true creatinine was 73 per cent (range 51 to 107 per cent) of the Jaffe chromogen in serum. The same data indicate that the renal clearance of endogenous Jaffe chromogen is, on the average, only 73 per cent of the GFR, so that, as determined by the method at hand, the Jaffe chromogen clearance is not a measure of the GFR. The data indicate that the non-creatinine component of Jaffe chromogen is either not diffusible through the glomeruli or is diffusible and subsequently reabsorbed completely by the renal tubules. The latter possibility is supported by the fact that in eight patients with accumulations of serous or edema fluid the mean concentration of endogenous Jaffe chromogen was 1.99 mg. per cent in serum water and 2.00 mg. per cent in other extracellular water.

of renal function by measurement of the serum concentration of creatinine must assume a normal output of creatinine from the muscles.

Since Shaffer's original studies (34) it has been stated by some observers that the *total* creatinine coefficient (mg. creatine expressed as creatinine + mg. preformed creatinine excreted per day/kg. body weight) in subjects with creatinuria equaled the creatinine coefficient in the normal (14, 22). This concept is difficult to reconcile with present knowledge of the differing modes by which the kidney treats creatine and creatinine. Since creatine is reabsorbed by renal tubules, while creatinine is not, equimolar loads of creatine and creatinine would not result necessarily in equimolar excretion of the two substances. On these grounds it would be anticipated that the *total* creatinine coefficient would be reduced in patients with a diminished creatinine coefficient and a spontaneous creatinuria. A review of reported data on the excretion of total creatine bodies by subjects with creatinuria reveals, in fact, that the total creatinine coefficients were below the normal range. For example, in seven adult males with progressive muscular dystrophy and copious creatinuria on a creatine-"free" diet, total creatinine coefficients ranged from 13.0 to 18.3, compared to the normal range of 20–26 (23). And in the two patients with obsolete anterior poliomyelitis reported here the total coefficients were less than those reported for normal women. It would appear, then, that insomuch that creatine is reabsorbed, the total creatinine coefficient in creatinuric patients will be less than the creatinine coefficient of normal subjects. And variations in reabsorption rate may underlie the inconstancy of the total creatinine coefficient reported by many observers.

These considerations render unproved the assumption made by some observers (17) that the sum of daily excretion of creatine, creatinine and guanidoacetic acid equals the amount of creatine synthesised.

SUMMARY

1) Creatinuria may be considered to be the result of an increased tubular load of creatine (serum creatine concentration × glomerular filtration rate), decreased tubular reabsorption rate, or a combination of both. On theoretical grounds, tubular load may be augmented by

acceleration of (GFR), by administration of creatine, accelerated synthesis of creatine, extrusion of intracellular creatine, or by inadequate disposition of creatine in the muscle cells.

2) The ability of the renal tubule to reabsorb creatine was reduced and creatinuria was observed (a) during prolonged administration of desoxycorticosterone acetate and in a patient with Cushing's disease, (b) during administration of thyroid substance in instances of hypothyroidism, and (c) during the puerperium. The creatinuria accompanying the administration of methyltestosterone to man had no renal tubular component.

3) In obsolete anterior poliomyelitis, creatinuria followed simple reduction in muscle mass, an example of inadequate bulk for proper disposition.

4) By isolation of some of the mechanisms concerned with the production of creatinuria, it appeared that the anomalous absence of creatinuria in patients with myotonic dystrophy may be the result of reduced synthesis of creatine.

5) The creatinine coefficients and the serum concentration of creatinine are functions not only of the active muscle mass but also of the glomerular filtration rate.

We are indebted to Miss Rachel Fee, Mrs. Lina Bell, Miss Eileen English and Miss Nancy McCarthy for nursing assistance; and to Miss Liana Cernoia and Miss Bess Crozier for Supervision of diets. We wish to express our appreciation to Dr. R. E. Lenhard and to Mr. H. O. Kendall who made possible the studies of poliomyelitis by arranging for our observation of these patients. Dr. E. V. Newman has been generous in his advice regarding several of the technics employed to assess renal function. Drs. N. J. Eastman, B. Williams and N. Long kindly permitted us to study the patients from the Obstetrical Service. Dr. M. M. Ravitch kindly brought to our attention his patient with the amputation of leg and hemipelvis.

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PRELIMINARY OBSERVATIONS ON THE EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) IN ALLERGIC DISEASES

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Received for publication September 26, 1949

In April, 1949 Hench and his collaborators (1) described the dramatic effect of cortisone and adrenocorticotropic hormone (ACTH) in patients with rheumatoid arthritis. In a subsequent report (2) suggestive results were obtained with cortisone in acute rheumatic fever. In one case ACTH was administered in small doses for a period of seven days. Fever subsided within forty-eight hours and articular symptoms within four days.

The experimental studies of Rich and Gregory (3, 4) suggested a relationship between the hypersensitive state and the rheumatic diseases. The possible usefulness of ACTH in the control of the hypersensitivity state was first explored in a patient with severe exfoliative dermatitis due to iodine. The unique rapidity of recovery in this critically ill patient stimulated the trial of ACTH in other allergic states.

In the second patient extensive giant urticaria, joint pains and fever associated with a "serum disease type" sensitivity to penicillin were abolished within twenty-four hours after ACTH was begun.

The prompt control of the chronic asthmatic state and the striking alterations in the tissues of the upper respiratory tract in five asthmatic patients form the basis of this preliminary report.

In two patients the asthma was thought due to combined external and intrinsic factors. In the other three it was of the intrinsic type. The age of the patients ranged from 26 to 63 years, and the duration of the asthma from 5 to 23 years. The sputum in all contained many eosinophils. In several of the patients no more than partial and very temporary relief was obtained by the administration of adrenalin, aminophyllin, and ether by rectum.

The initial daily dose of ACTH varied between 30 and 100 mgm. given intramuscularly, divided in equal portions at six hour intervals. Unequivocal benefit was noted in from four to forty-eight hours. Complete freedom from all asthmatic symptoms occurred within one to eight days. In four of the patients there was total disappearance of sputum, and abnormal physical signs in the chest. In the fifth patient, who was six months pregnant, rhonchi persisted although she was free of asthmatic symptoms. Coincident with symptomatic relief, tracings of the expiratory phase of respiration showed improvement with removal of the relative obstruction to outflow.

Treatment was maintained for eleven to twenty-one days. The daily dose was gradually reduced after clinical recovery; the total amount administered ranging from 360 to 775 mgm. One patient has remained asymptomatic for one month after therapy was stopped.

Detailed examinations of the upper respiratory tract, including nasopharyngoscopy, were made on four of the patients. Three patients had a pale, edematous, polypoid nasal mucous membrane which was bathed with a thick mucopurulent discharge. During therapy the membrane became bluish pink in color, was covered with clear mucus, and the edematous, polypoid appearance was no longer present. The breathing space was greatly enlarged. The lymphoid tissue in the nasopharynx, which was covered with a thick discharge, was pale and edematous. During treatment the edema subsided, an orange pink color developed, the crypts became more prominent, and it was easily outlined from the surrounding mucous membrane. There was no gross change in the volume of the lymphoid tissue present. In two patients there was complete obstruction of the nose by polyps. These began to shrink on the third day of treatment and by the end of therapy had completely vanished in one and almost completely in the other. The three cases with mucous membrane abnormalities had antral clouding on roentgenological examination which cleared during the administration of ACTH. Several small polyps had reappeared in one patient on the twenty-third day after cessation of therapy.

The intradermal reactions to inhalant and bacterial antigens were followed in three patients. Two showed marked skin sensitivity to pollens and other extrinsic antigens. In one the sensitivity was greatly diminished during treatment but returned to its original level three weeks after cessation of ACTH. In the other patient no alteration was

observed. In both patients serum reagin titration revealed no change. The intradermal reaction to bacterial antigens decreased significantly in two patients and did not change in another. In one the sensitivity has returned to the pretreatment level three weeks after discontinuance of ACTH.

The previously described (1, 2) metabolic effects of ACTH in no instance were a serious complicating factor in these cases.

These clinical studies suggest that ACTH may have an important action in blocking various hypersensitivity reactions. Further observations are necessary to establish its efficacy in allergic states, and the effects of ACTH in other types of hypersensitivity are under study.

The ACTH used in these studies was supplied by Armour and Company through the courtesy of Dr. John R. Mote.

We wish to thank Dr. Walter Baetjer, and Dr. Milton Sherry for permission to treat patients under their care.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

The Commonsense Psychiatry of Dr. Adolf Meyer. By Alfred Lief. XVIII + 677 with a frontispiece, glossary and index. *McGraw-Hill, New York*, 1948. $6.50.

Under this title, Mr. Lief has presented an anthological biography of Dr. Adolf Meyer,—a book, largely in Dr. Meyer's own words, in which one can trace the development of his thought and teaching, with sufficient biographical material to provide personal perspective.

Large numbers of students and associates who knew Dr. Meyer as "the Professor," have now this solid, attractively printed, volume with which to re-view many matters originally viewed as difficult or incomprehensible. In this review many will find themselves a bit puzzled to understand why it was ever so mystifying. Mr. Lief has wisely chosen those central themes of Dr. Meyer's developing thought around the ever-recurring, deeper problems of psychiatry and medicine, and those who now re-read his words, with a mature medical experience as background, may therefore find them more understandable, more exciting, more cogent, than they seemed when originally heard or read.

Another large group of readers will also find this an extremely useful and stimulating book—the great number of present-day psychiatrists to whom Dr. Meyer has been a symbol often referred to but not so often read. Even those who have spoken or written glibly about "Adolph Meyer" and who profess to have read his (non-existent) "textbook of psychiatry" can now achieve an approximate honesty by reading this book.

Your reviewer, in reading Mr. Lief's book, has gained a clearer perspective of Meyer as essentially a biologist with a special interest in constructing a broad basis for a biological "science of man." For this general purpose, comparative neurology was Meyer's "structural-functional" framework, and in clinical psychiatry, "the experiments of nature" were daily "scrutinized" for better understanding of "man-function." The ergasia-formulations may be taken symbolically to represent Dr. Meyer's preference for a dynamic biological (Greek-rooted) functional frame of reference, and his aversion to a static (Latin-rooted) pigeon-holing orientation for clinical psychiatry.

The title to Mr. Lief's book somewhat unpleasantly implies a proprietary claim to "common sense," as if other psychiatric views and practices did not partake of "common sense." Your reviewer might undertake to dispute the aptness of this implication if it were not so difficult to define "common sense" when used as a laudatory adjective. There is a deeper meaning, however. The "consensus" was an inherent part of Dr. Meyer's mode of operations. He sought a "consensus" with each patient; he sought a "consensus" with his staff; with other earnest work-

ers in the broad fields of psychiatry and neurology; and this everlasting search for common ground and mutual agreement was an outstanding characteristic of Meyer's work pattern—the Meyergasia, so to speak.

In this sense of a searching for the sense of things, and searching for a sense which could be agreed upon in common, "common sense" is an apt methodological characterization of Adolf Meyer's method of psychiatric work.

JOHN C. WHITEHORN

Synopsis of Psychosomatic Diagnosis and Treatment. By FLANDERS DUNBAR, M.D. 501 pages. *C. V. Mosby Company, St. Louis,* 1948.

Dr. Dunbar's reputation, the excellent bibliography, and the handy format make this book very tempting. However, as poor organization, poor selection of material, and faulty editorship are the outstanding features; the reader's attention must be directed to the excellent chapter on metabolic and endocrine disorders written by Dr. Robert C. Lowe, and the highly competent portions devoted to gastrointestinal disorders and arthritis.

The book is not a synopsis. It is a collection of original articles mixed with a peculiar array of incomplete chapters. Some topics are covered in considerable detail while other important subjects are either mentioned in passing or are entirely omitted. Some chapters have little place in a book with this title and were better eliminated, particularly when it has been found necessary to confine all remarks on general therapy and rehabilitation to four pages.

The book is introduced by a chronology of fetal development; its pertinence is purely hypothetical and of little interest to the reader of a synopsis. The second chapter by L. W. Sontag is an intriguing and scholarly presentation of determinants of predisposition to psychosomatic ailments; the emphasis on genetic and intrauterine influences is not only purely hypothetical, but out of keeping with most of the literature including the chapters which follow in the book. The third introductory chapter concerning the nervous system seems to the reviewer to be a poor and uncritical selection of data.

The editorship of the clinical chapters is puzzling. For example, the chapter entitled "Vasomotor, Trophic, Oral, and Skin Disorders" confines itself to skin ailments and completely neglects such conditions as Raynaud's Syndrome, migraine, and causalgia. The chapter on "Eye, Ear, Nose, Throat, and Dentition" devotes twenty pages and the only illustrations in the volume to disorders of dentition, most of which is not only trivial but of little interest to the internist; but does not mention the nose and throat, although the author must be aware of the work on emotional influences on the nasal mucosa being carried on at Cornell. There are many other strange inconsistencies.

The book can be recommended for the few good chapters with the hope that the remainder will not turn the reader from the field which Dr. Dunbar had previously done so much to further.

T. L.

Geriatric Medicine. The Care of the Aging and the Aged. 2nd Edition. E. J. STIEGLITZ, EDITOR. 773 pp. *W. B. Saunders, Philadelphia*, 1949. $12.00.

Forty-seven experienced authors have contributed 44 chapters to this text-book which covers the fields of medicine and some aspects of special surgery. The aim of this symposium is to enhance the "application of understanding of the changes which come with senescence to all the facets of medical practice". The need for such an effort is beyond argument; there is some ground, however, to question whether a text of this nature is the best solution to the problem.

There are some introductory chapters on general principles of the aging process which make available a certain number of valuable quantitative data diluted by a large quantity of generalisations, aphorisms and slogans which are presented in extended form. In some sections the paucity of specific information is disappointing; in others, many clinicians would disagree vehemently with the advice given. For example, in a brief discussion of sleep, the only sedatives mentioned are phenobarbital and sodium pentothal; failure to mention barbital and chloral hydrate is difficult to understand when the drugs recommended here are notorious for their capacity to produce confusion in the elderly patient.

A second criticism may be directed toward the inclusion in this book of articles which, although superb clinical treatises, are not of special geriatric interest. For example, Talbott's chapter on gout and Keefer's on diseases of the aorta are magnificent essays which appear to be forced into the book for purposes of completeness. In view of the rapidly mounting costs of medical texts, it would seem important that authors and editors strive to reduce rather than expand the size of their books.

It is somewhat dismaying to find in a text of 773 pages devoted to geriatric medicine that "Mental Changes with Normal Aging" is dealt with in a matter of 14 pages and "Mental Disease" in another 15 pages. By contrast, "Surgical Renal Disease" occupies 16 pages, "Diseases of the Prostate" 18 pages and "Diseases of the Bones" 31 pages.

Despite certain deficiencies noted, this text provides in one source a large quantity of information related to geriatric medicine which is disseminated throughout the literature. It will furnish a starting point for those requiring an introduction to gerontological problems and is to be recommended as a preliminary reference text.

J. L. L., Jr.

Cardiac Catheterization in Congenital Heart Disease. ANDRE COURNAND, JANET S. BALDWIN AND AARON HIMMELSTEIN. 108 pp. $4.00. *Published by the Commonwealth Fund, New York.*

The book of Cournand and his co-workers is primarily concerned with noncyanotic malformations of the heart. Its general section deals with the technique, equipment and with complications of the method. This section also includes characteristic blood pressure tracings and formulae used in the calculation of the blood

flow through various parts of the circulation. Cournand is well equipped to discuss this procedure since he was the first to use catheterization as a diagnostic tool in this country, and to standardize the technique so that it may be safely used for diagnosis and investigative purposes. Several points in the general section of the book deserve mentioning. The authors perform catheterization in children under 9 years of age under basal avertin anesthesia. Other workers prefer morphine-scopolamine. Cournand and his co-workers use a modified Hamilton manometer and also mention the more popular electrical recording devices. According to Cournand, the most frequent complications of catheterization in Congenital Heart Disease are, air emboli, venous and intracardiac thrombosis and cardiac arrythmias. The latter is the most serious complication. Fortunately, however, the combined deaths from the procedure, reported from various laboratories is probably less than .1 percent. The pressure recordings are excellent and should be of the greatest value to all those who are interested in the diagnosis of Congenital Heart Disease. The formulae for the calculation of the various volume flows are similar to those used in other laboratories.

In the second section of the book Cournand and his co-workers deal with the application of catheterization to specific malformations of the heart and great vessels. The correlation of clinical and physiological findings is of particular value, since it enables the authors to establish patterns for the more typical forms of Congenital Heart Disease.

Cournand's book stresses the fact which has been so often overlooked in the past that a similarity in gross pathology is often associated with a large variety of physiological and clinical findings. The book should be considered essential to all those who are interested in the application of modern physiological methods to the study of Congenital Heart Disease.

RICHARD J. BING

Practice of Allergy. By WARREN T. VAUGHN, M.D. Revised by J. Harvey Black, M.D., Second Edition, 1132 pp. Illus. $15.00 *C. B. Mosby Company, St. Louis*, 1948.

The present writer had the opportunity of reviewing the first edition of this book published in 1939. The second edition necessitated by ever-increasing knowledge in the allergic field was interrupted by Dr. Vaughn's untimely death; and Dr. J. Harvey Black undertook the difficult task of completing the revision. Dr. Black tried, as he states, to "retain the quality and the flavor of the book so that it might remain as it was written—Warren Vaughn's book." Except for completely new and good sections on pollen field surveys and the development and technique of Aerobiology by O. C. Durham, Chief Botanist of the Abbott Laboratories; a complete revision of the chapter on fungus infection and associated allergies by Dr. J. B. Howell; and a chapter on vital capacity and pulmonary physiology by Dr. James Holman, the general text remains essentially as Dr. Vaughn originally wrote it in 1939.

The historical aspects of the development of our present understanding of

clinical allergy and the discussion of the general characteristics of clinical allergy are followed by individual sections devoted to the physiology of allergy, allergic diagnosis, diagnosis and treatment of food allery, food allergens, pollens and other inhalent antigens, bacterial allergy, fungi, serum disease, anaphylactic shock, drug allergy, contact allergy, physical allergy, the pharmacology of allergy and finally the specific allergic syndromes as met with in human beings.

Dr. Vaughn possessed an encyclopedic mind and the subject matter is presented in an interesting manner. His style, however, is somewhat rambling, repetitious and at times speculative in character. Dr. Vaughn was particularly interested in special phases of allergy which are given a disproportionate amount of space. One hundred and sixty-one pages are devoted to the diagnosis and treatment of food allergy, the importance of which in clinical allergy has been seriously questioned by many physicians in recent years. Food allergy as a common cause of asthma, chronic rhinitis, migraine, colitis and hypertension has aroused skepticism in many minds. Of the prominent allergists in this country, Dr. Vaughn was an expert botanist and the detailed section of two hundred and sixty-eight pages devoted to plants and pollens reflect his interest and knowledge.

The treatment of hay fever of the seasonal type reflects the fact that the immunologic mechanism conferring protection on the individua is not yet known; and therefore, the appropriate dose of pollen extract for any given individual cannot be foretold. The discussion of perennial rhinitis, a more serious affliction though less common than seasonal hay fever in which aetiology and successful therapy is more difficult to obtain receives only minor consideration. Likewise, the section on urticaria and angioneurotic oedema, especially of the chronic type should be amplified. Only twenty-four pages are devoted to asthma where as thirteen pages are devoted to migraine, which in the experience of this reviewer is only rarely due to food allergy. The disproportionate emphasis on special subjects should be eventually corrected.

Certain sections could be combined to prevent repetition; for example, anaphylactic shock and serum disease could be included in the first section which covers the historical development of anaphylaxis and clinical allergy. The section on clinical characteristics of allergy (Part II) could be combined with allergic diagnosis (Part IV). There is, however, much good material in this book reflecting Dr. Vaughn's wide clinical experience as stated in the review of the first edition ten years ago. The many case analyses, the numerous good photographs taken by the author with a Lecca camera lend a personal touch not usually encountered in most text books. Numerous portraits of early investigators are also of interest. The bibliography is extensive but not complete. The printing, on good grade paper, is easy to read, a recommendation when one considers the size of the book.

A textbook of over 1000 pages on a special phase of medicine necessarily must contain a great amount of detail, to the extent that this volume serve more as a reference book than a concise text book for medical students and physicians who are not too familiar with the minutae of allergy.

W. L. W.

Advances in Pediatrics, vol. 3. Edited By S. Z. LEVINE and others 363 pp. $7.50. *Published by Interscience Publishers, Inc. New York.*

This volume contains eight excellent articles written by experts. Five of the articles are factual reviews of the subjects covered. These are of timely interest and a storehouse of information to be used either for teaching or for meeting a specific practical problem in diagnosis or therapeutics. The other three articles are of a different nature, for in them the contribution is that of point of view of the author himself. All three deal with some phase of development. Lawson Wilkins' article deals with sexual development, and although much is concerned with physiological and anatomical abnormality, there is an excellent discussion of the variations in "normal" development, a discussion much needed in these days of commercialized pressure to use endocrine drugs of doubtful efficacy or safety, whenever the patient varies even a little from the average.

Hilde Bruch's article on puberty and adolescence is an original contribution of first rate importance, amounting to much more than a review of recent advance. In it, she has reviewed an entire subject including anthropological, sociological and other aspects of puberty, as well as making her own contribution to an understanding of the psychology of this period.

Milton Senn's article while in general a good presentation of the psychological factors in childhood development, tends too much to dogmatic statement. He illustrates a fault not too uncommon among psychiatrists of generalizing on the basis of "slanted material"—the psychiatrist seldom sees a "normal" child, except his own. In spite of these somewhat minor "defects" the article is a good presentation of present points of view in child psychology.

H. W. J.

Hematology for students and practitioners. Revised. 2nd edition. WILLIS M. FOWLER. 535 pp. $8.50. *Paul B. Hoeber, Inc., New York, N. Y.*

This compact volume is designed to present in concise form a resume of clinical hematology for the medical student and practitioner. The major portion of the text is concerned with descriptions of the blood dyscrasias. However, there are brief sections dealing with basic anatomical and physiological concepts and a chapter describing routine hematologic methods. The volume is not intended as a reference work and does not deal exhaustively with the subject matter discussed. A bibliography is provided at the end of each chapter, but specific references to the literature are not cited in the text. The book is clearly written, is very readable and contains a wealth of accurate and well condensed information. The author presents fairly both sides of many controversial points, but here and there unqualified statements appear which certainly would not meet general approval. For example, the normal intravascular fluidity of the blood is attributed to the presence of heparin. In another section, one is led to believe that leukemia can be transmitted from man to animals. In view of our present knowledge of iron me-

tabolism it seems regrettable that the term "idiopathic" hypochromic anemia has been retained. For the most part, however, the book is up-to-date and is very adequate for the purpose for which it was written.

C. L. C.

Clinical Cystoscopy. By LOWRAIN E. MCCREA. 2nd ed. 1152 pp. 742 Illus. (201 in color) $28.00. *F. A. Davis Company, Philadelphia*, 1949.

This is an enlargement of an already proven first edition authoritative text on cystoscopy. There is a presentation of every phase of cystoscopy and related urological fields so that in fact it becomes almost an omnibus of urology. The two two volumes are beautifully printed, and the illustrations on almost every page add clarity and understanding to the well presented and well documented text. The enlarged and improved index and the profuse bibliography are helpful in attaining the desired end—a ready, useful reference book.

The author's use of his excellent cystoscopic photographs, in which he has pioneered, is interesting and instructive. However, the cystoscopic drawings are often clearer, and one wonders if a more careful use of post mortem material might not be more satisfactory. The text and value of the book often suffer from the attempt to include a little bit about everything in cystoscopy and related urology, sometimes at the expense of fundamental detail. At times the author is of necessity didactic and sometimes on controversial subjects. Unfortunately, some of the drawings, which are copies of familiar originals suffer in the transfer; and one wonders why the originals were not used.

The two volumes undoubtedly cover the field of clinical cystoscopy more completely and carefully than any other available text. Their breadth of scope and their usefulness as reference material for the unusual as well as the commonplace findings of cystoscopy should recommend them highly to any student of urology and particularly to the neophyte who is trying to master the intricacies of the subject.

W. E. G.

Atlas of Peripheral Nerve Injuries. WILLIAM R. LYONS AND BARNES WOODHALL. 339 pp. $16.00. *W. B. Saunders, Phila. Pa.*

This monograph presents the pathologic changes of peripheral nerves as they have been seen in war casualties of World War II treated in Army general hospitals during the years 1943, 1944 and 1945.

After a brief summary of the pathologic techniques used in the preparation of the tissues, and a glossary of the terms employed, the authors describe briefly the normal histological structure of peripheral nerve. The incidence of periphery nerve injuries in World War I and II is discussed with particular reference to the surgical treatment.

By means of hundreds of photographs, some in color, of gross and histological specimens, the authors illustrate the changes in the proximal and distal stumps of

severed nerves, the alterations in neuromas in continuity, and the effects of ischaemic lesions on the peripheral nerve and muscle. The outstanding feature of this book is the profuse use of illustrations with accompanying legends and pertinent comments of interest to all workers in the field of neurology.

Although a representative bibliography is appended, little attempt is made to integrate the contents of the monograph with pre-existing knowledge of peripheral nerve pathology. Perhaps the voluminous material at the disposal of the authors seemed sufficiently great to make superfluous, comments on opinions based on fewer cases. Many readers will wish that the literature had been more fully discussed.

As an atlas, this is a superb contribution. Its format and printing are pleasing to the eyes. But one could not help wishing that the end results in the cases had been known, so that the effect of the various pathological states described might be correlated with the functional restoration of the nerve. We may hope that the exigencies of civilian life do not place too many obstacles in the way of the authors, so they may prepare, as they announce in an addendum, a separate monograph on the influence of the neuropathologic changes described in this atlas upon peripheral nerve regeneration.

A. E. W.

BOOKS RECEIVED FOR REVIEW

A Textbook of Neuropathology. BEN. W. LICHTENSTEIN. 474 pp. $9.50. *W. B. Saunders, Phila. Pa.*

Change of Life. EMIL NOVAK. 127 pp. $2.00. *Women's Press, New York.*

Diagnosis of Pancreatic Disease. LOUIS BAUMAN. 74 pp. $5.00. *J. B. Lippincott, Phila. Pa.*

Epilepsy and Convulsive Disorders in Children. EDWARD M. BRIDGE. 670 pp. $8.50. *McGraw-Hill Co., New York*

Mental Hygiene in Public Health. PAUL V. LEMKAU. 396 pp. $4.50. *McGraw-Hill Co., New York*

Autobiography of Dr. Robert Meyer. EMIL NOVAK. 126 pp. $2.50. *Henry Schuman, New York.*

Morgagni's Syndrome. FOLKE HENSCHEN. 172 pp. 30/ net. *Oliver & Boyd Ltd., Tweeddale Court, Edinburgh*

Radiologic Exploration of the Bronchus. S. DI RIENZO. 332 pp. $10.75. *Charles C. Thomas, Springfield, Illinois.*

Shearer's Manual of Human Dissection, 2nd edition. CHARLES E. TOBIN. 286 pp. $4.50. *The Blakiston Co., Phila. Pa.*